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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Simplified food webs lead to energetic bottlenecks in polluted lakes

Graham D. Sherwood, Jennifer Kovecses, Alice Hontela, and Joseph B. Rasmussen

Abstract: Very little is known about the consequence of human activities on the flow of energy through natural ecosystems. Here, we present a trophic-based approach to describing energy relationships in pollutant-disturbed lakes, emphasizing the importance of prey diversity in maintaining energy transfer to growing fish. Both diet and community analysis indicated that the food web leading to yellow perch (*Perca flavescens*) in metal-polluted lakes was extremely simplified compared with reference lakes. Through the application of an in situ marker for fish activity costs (muscle lactate dehydrogenase activity) and through bioenergetic modelling, we show how this has severe consequences on the efficiency of energy transfer to perch from their prey; premature energetic bottlenecks (zero conversion efficiency) occur when successively larger prey types are not available to growing perch. These observations provide a much needed ecological and physiological framework for assessing how energy transfer can be affected in polluted systems. Our approach need not be limited therein but should be applicable to any aquatic system where food web structure is variable and (or) disrupted.

Résumé : Très peu est connu au sujet de la conséquence des activités humaines sur le transfert énergétique dans les écosystèmes. Cette étude présente une nouvelle approche pour décrire des relations énergétiques dans les lacs pollués, soulignant l'importance d'une diversité de proies pour la croissance des poissons. Les analyses de contenus stomacaux et de la communauté ont indiqué que la base de nourriture disponible aux perchaudes (*Perca flavescens*) provenant de lacs pollués par les métaux lourds a été extrêmement simplifiée comparativement aux lacs références. Par l'application d'un indicateur enzymatique pour les coûts d'activité (natation) chez les poissons, et par l'application d'un modèle bioénergétique, cette étude démontre que ceci a des conséquences graves sur l'efficacité du transfert énergétique de proies aux perchaudes; des entonnoirs énergétiques (aucune croissance) se produisent quand des proies de différentes tailles ne sont pas disponibles successivement aux perchaudes en croissance. Ces observations fournissent un cadre écologique et physiologique important pour évaluer comment le transfert énergétique peut être affecté dans les systèmes pollués. Cette approche n'a pas besoin d'être limitée spécifiquement aux systèmes pollués, mais pourrait être aussi appliquée à tous systèmes aquatiques où la chaîne alimentaire est variable et (ou) dérangée.

Worldwide, species are being lost at an alarming rate. In North America alone, 123 freshwater animal species have been recorded as extinct in the past 100 years and many more are presently in peril (Ricciardi and Rasmussen 1999). Aside from the species-conservation issues that this raises, there are some concerns regarding the inevitable restructuring of natural food webs and the effects that this may have on ecosystem function and stability (McCann 2000). Indeed, a growing body of evidence suggests that food-web structure is vulnerable to a wide array of human activities, including species introductions or invasions (Vander Zanden et al. 1999), habitat alteration (Wootton et al. 1996), and environmental warming (Petchey et al. 1999). Our ability to predict how ecosystem function will respond to these pressures will require a solid understanding of how energy flows within natural food webs. To this end, and in terms of tracing pathways of energy flow, stable isotopes have proven invaluable (e.g., Vander Zanden et al. 1999). However, a more com-

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G.D. Sherwood,[1] J. Kovecses, and J.B. Rasmussen. Department of Biology, McGill University, 1205 Dr. Penfield, Montreal, QC H3A 1B1, Canada.
A. Hontela. TOXEN Research Centre, Université du Québec à Montréal, C.P. 8888, Succ. Centre-ville, Montréal, QC H3C 3P8, Canada.

[1]Corresponding author (e-mail: grahamsherwood@hotmail.com).

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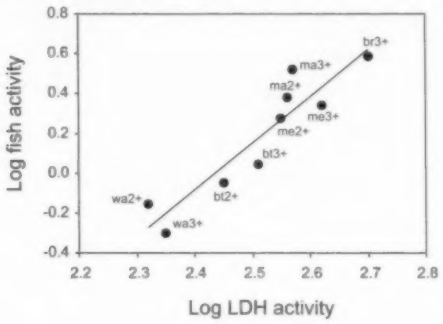
plete understanding will clearly necessitate a capacity to evaluate the efficiency of energy transfer among trophic compartments and how this may vary within and among natural and disturbed food webs.

Here, to illustrate this point, we show how heavy-metal loading into lakes (from copper-smelting operations) alters food-web structure and eventually leads to a blockage of energy transfer to growing fish. Our approach combines physiological measurements, diet and community analysis, and bioenergetic modelling. The first step of our investigation involved measuring the activity of the glycolytic enzyme, lactate dehydrogenase (LDH), in the muscle of yellow perch (*Perca flavescens*) from five non-metal-impacted populations with varying levels of predetermined fish activity (*A*, swimming) costs (Boisclair and Leggett 1989). We observed a strong, positive relationship between muscle LDH activity and fish activity (Fig. 1; $r^2 = 0.86$, $p < 0.001$, $n = 9$; $\log A = 2.35 \log \mathrm{LDH} - 5.70$). This result was not surprising as LDH is involved in supplying quick, burst energy to spontaneous movements (Somero and Childress 1980), such as would be necessary for attacking prey when feeding, and its activity in the muscle has been suggested to be related to patterns of locomotory activity in at least one other study (Somero and Childress 1980; see also Sherwood et al. 2002). Measurements of LDH activity may provide a snapshot of recent (days–weeks) energetic requirements; its activity in fish has been shown to respond to altered energetic demands within at least, but not earlier than, 7 days (Schulte et al. 2000).

We then applied this enzymatic marker to inferring patterns in fish activity in perch from four lakes along a gradient of metal contamination. LDH activity scaled positively to body size in all cases (Fig. 2*a*), as has previously been noted (Somero and Childress 1980). In the case of the two reference lakes, the mass-dependent rise in LDH activity appeared to break suddenly between 15 and 20 g and again at about 200 g, creating a distinct "saw-toothed" pattern. The timing of the first break corresponded to a well-defined shift in feeding habit (zooplanktivory to benthivory, Figs. 2*b*, 2*c*) and would have involved a drop in fish activity of about twofold, an enormous savings in terms of a fish's energy budget. The second break occurred around the point where perch became almost entirely piscivorous (Fig. 2*b*) and would have meant an even larger savings. We interpret these sudden drops in LDH activity as a function of foraging costs; attack rate, in terms of number of feeding bursts, will be high for fish foraging on disproportionately small prey, and it would appear that perch shift to progressively larger prey when the cost of fish activity gets prohibitively high. Alternatively, shifts to larger prey could result from overcoming gape limitation. No distinct diet shifts or associated saw-toothed patterns in LDH activity were evident for either of the two polluted populations; although both stable isotope ($\delta^{13}C$) and stomach content analyses suggested an important contribution of chironomids to the diet of lake Osisko perch (Figs. 2*b*, 2*c*), it was unclear where exactly this shift from zooplanktivory occurred (chironomids are a prey type that perch normally avoid in favour of attacking larger, more accessible taxa such as ephemeropterans; G. Sherwood, unpublished data). Finally, LDH activity diverged quite early so that it was always higher in the two polluted populations than in the two reference populations (Fig. 2*a*). It is possible

Fig. 1. Log muscle LDH activity (absorbance units per minute per milligram white muscle protein) versus log fish activity (unitless) for yellow perch from five non-metal-impacted Eastern Townships populations; br, Lake brome; bt, Lake Bromont; ma, Lake Magog; me, Lake Memphremagog; wa, Lake Waterloo; suffix "2+" or "3+" refers to age-class. Fish activity values are from Boisclair and Leggett (1989).



that this was related to a higher dependence on smaller, faster-moving zooplankton (i.e., copepods) in metal-polluted lakes; the numerical ratio of copepods to larger-bodied cladocerans in the diet of zooplanktivorous perch was 2:1 in metal-polluted lakes compared with 1:3 in reference lakes. This may reflect higher predatory pressures on "easy-to-catch" zooplankton (cladocerans) in polluted lakes where diet options following zooplanktivory are limited.

The final step of our analysis involved fitting lines to the observed LDH activities versus body size and inserting the appropriate slopes, intercepts, and breaks for related fish activity into a bioenergetic model (Kitchell et al. 1977), which can then be used to estimate conversion efficiency based on body size (Fig. 3). It should be noted that fitting lines in a manner consistent with observed diet shifts, as opposed to simple linear regression techniques, led to a considerable improvement in tightness of fit between LDH activity and body size (Fig. 2*a*). The overall result of our modelling exercise was to show that perch conversion efficiency was always lower in polluted lakes than in reference lakes. Perch from reference lakes continued to grow more efficiently because they had the option of resetting fish activity by periodically switching to larger prey types. Perch from the two metal-polluted lakes could not do this. They had either no prey choice (Lake Dufault) or very limited choice (Lake Osisko) following zooplanktivory (Figs. 2*b*, 2*c*, 3*b*(inset)); a more in-depth analysis (J. Kovecses, unpublished data) suggests that this was a result of metal toxicity whereby most benthic invertebrates with close association to contaminated sediments were removed from the community. As a result, both Lake Dufault and Lake Osisko perch could not benefit from the energetic advantages of switching prey, and their growth bottlenecked at comparatively small sizes (Fig. 3). This may explain why perch from both metal-polluted lakes, unlike their reference counterparts, never reached piscivory. For Lake Dufault, this did not seem to be related to low prey-fish availability. In fact, our seining efforts have revealed considerable densities of common prey fish such as spottail shiners (*Notropis hudsonius*) in this lake. Rather, it seems that Lake Dufault perch were deprived of the benthi-

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Fig. 2. (*a*) Muscle LDH activity vs. body size in Abitibi perch from four lakes along a gradient of metal contamination. For reference perch, log LDH = 0.49(0.04)·log*W* + 1.97(0.03) – 0.30(0.06) – 0.56(0.10) (r^2 = 0.89, p < 0.0001, n = 45; all model terms were significant to p < 0.0001; area of diet overlap (10–30 g, shaded area spanning all panels) was not included in regression; for model without diet shifts, r^2 = 0.75; values in parentheses are standard error). For Lake Osisko perch, log LDH = 0.30(0.02)·log*W* + 2.30(0.04) (r^2 = 0.85, p < 0.0001, n = 25; no diet shifts were considered). For Lake Dufault perch, log LDH = 0.53(0.04)·log*W* + 2.11(0.04) (r^2 = 0.96, p < 0.0001, n = 12; no diet shifts were considered). (*b*) Perch diet expressed as range of perch body size for various diet stages. Approximate prey size ranges for prey types are in parentheses: "zooplankton" includes copepods and cladocerans; "insects" includes all aquatic insects, plus amphipods, other than "odonates" (which have their own category because of their large size); and "fish" is mostly young-of-the-year yellow perch and spottail shiner (*Notropis hudsonius*). (*c*) $\delta^{13}C$ vs. body size for Abitibi perch populations. More negative values (~–30‰) are indicative of pelagic feeding (i.e., zooplankton), and more positive values (~–24‰) are indicative of littoral feeding (i.e., benthic invertebrates and littoral fish) (Vander Zanden et al. 1999).

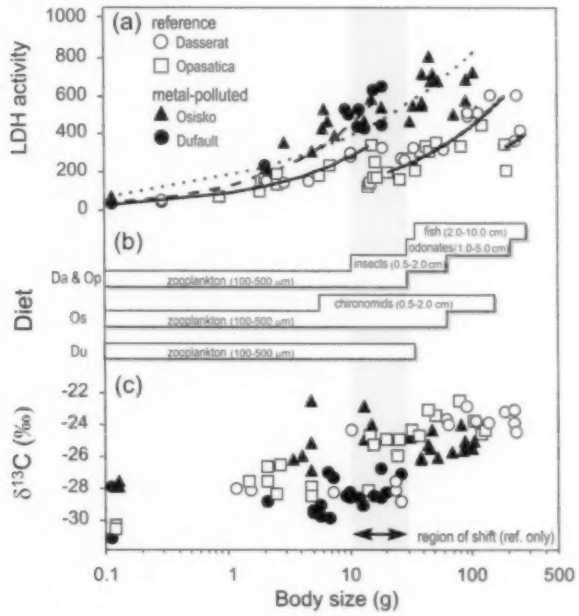
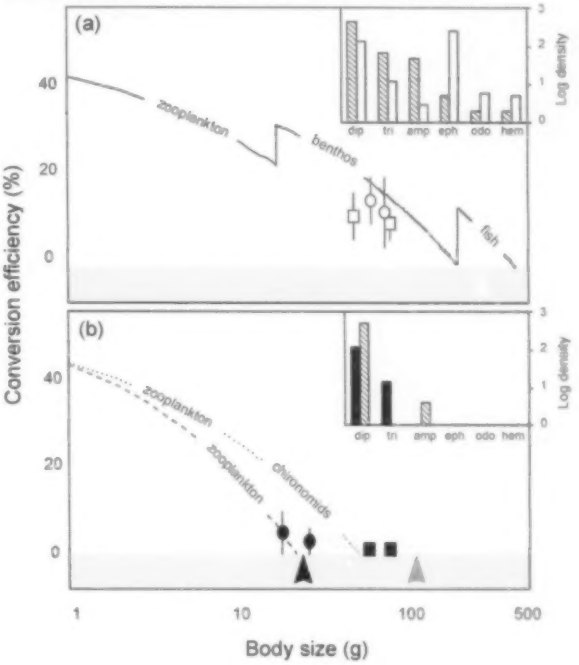


Fig. 3. Bioenergetic modelling results for conversion efficiency of (*a*) reference (Lake Dasserat and Lake Opasatica combined; solid line) and (*b*) metal-impacted (broken line for Lake Dufault and dotted line for Lake Osisko) perch in relation to body size. Arrowheads represent maximum observed body size for reference (open), Lake Dufault (solid), and Lake Osisko (shaded) perch. Individual data points are conversion efficiency values for age-4+ perch obtained from independent, in situ, growth and consumption estimates (Sherwood et al. 2000; open circles are for Lake Dasserat, open squares for Lake Opasatica, solid circles for Lake Dufault, and solid squares for Lake Osisko; vertical bars represent 95% confidence interval). Inset graphs are average density (individuals/m^2) of dipterans (dip), trichopterans (tri), amphipods (amp), ephemeropterans (eph), odonates (odo), and hemipterans (hem) in the littoral zone of Lake Dasserat (hatched bars, panel *a*), Lake Opasatica (open bars, panel *a*), Lake Dufault (solid bars, panel *b*), and Lake Osisko (hatched bars, panel *b*).



vory stage that provides the crucial "stepping-stone" to piscivory (i.e., they bottlenecked at sizes too small to become piscivorous). Lake Osisko perch, on the other hand, may have never become piscivorous because no other fish species are present in this lake and availability of small young perch is low.

Our approach is unique in that it combines both physiological and ecological principles and is simple in that it involves only a minor, yet important, modification to existing bioenergetic models (i.e., variable fish activity costs). In fact, fish activity has already been found to be highly variable among species (Rowan and Rasmussen 1996), populations (Boisclair and Leggett 1989), and stages of maturity (Rowan and Rasmussen 1996). Field measurements of LDH activity can provide a fast and effective way of evaluating this important and often neglected energetic variable in wild-living fish.

The results of this study underscore the importance of understanding possible indirect (i.e., food web) effects of toxicity on fish bioenergetics (e.g., fish activity rates) when collecting and interpreting field data in ecotoxicological studies. In a more general sense, our findings should have application to any aquatic system where natural feeding relationships are disrupted. For example, stable isotopes showed that bass invasion into Canadian-shield lakes forced indigenous lake trout to feed much more heavily than usual on zooplankton (Vander Zanden et al. 1999). Final trout size in zooplanktivorous versus piscivorous populations from the

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same system of lakes is almost an order of magnitude smaller (Ivano Pazzia, McGill University, Montreal, Quebec, unpublished data), suggesting that invasion-impacted lake trout are caught in a bass-induced energy bottleneck. It is these types of observations, coupled with what we observe here, that provide a compelling argument for the conservation of aquatic food webs in as much their natural state as possible. The trophic linkages that have evolved over time (and continue to develop through diet ontogeny) may represent the most energetically efficient pathways of energy flow through natural food webs.

Methods

Fish capture and diet analysis

Yellow perch were sampled in June of 1998 for five Eastern Townships populations (southern Quebec, 45°N, 72°W; these populations are all nonpolluted) and in June of 1999 for four Abitibi populations (northwest Quebec, 48°N, 79°W; these populations are at the extremes of a metal-contamination gradient (Cd, Cu, and Zn), Sherwood et al. 2000). Fish were captured by seine, gill net, and hook-and-line. Fish were sacrificed within 1 h of capture, and a cross-sectional sample of white muscle tissue was dissected from the tail section and immediately stored in liquid nitrogen for enzyme analysis.

Diet and community analysis

Stomachs were removed from frozen perch carcasses and contents were identified to order. Additional data for Abitibi lakes was available from previous years. The total number of full stomachs examined for Abitibi populations was 256 and never less than 40 per lake. Independent invertebrate sampling of the littoral zone of Abitibi lakes was carried out in June of 1999 by kicknet (8 samples per lake; 0.5 m^2 per sample). Individuals from each sample were counted and identified to order. Only those taxa that formed a major part of the benthic diet of perch were considered.

Enzyme analysis

Lactate dehydrogenase assays were performed on frozen white muscle tissue according to kit specifications (LDH-Optimized, Sigma Diagnostics®, St. Louis, Mo.) following a sample preparation protocol described in Somero and Childress (1980) with some modifications. Before homogenisation (using a rough glass and Teflon apparatus) in buffer solution (50 volumes; 0.07 M pH 7.0 KPO_4 buffer + 1 mM EDTA + 0.2% v/v Triton X-100), muscle samples were crushed to a fine powder in liquid nitrogen. This allowed for a more complete homogenisation. Fifteen microlitres of sample preparation, versus the 100 µL prescribed by Sigma Diagnostics®, were sufficient to initiate and sustain the LDH reaction for a duration of 1 min for the entire range of LDH activities encountered. LDH activities, measured at 20°C and expressed as arbitrary absorbance units (at 340 nm), were normalized to protein concentration to account for variations in homogenized tissue content from one sample preparation to another as well as variations among individuals. Protein analysis was carried out on sample preparations according to BioRad® (Hercules, Calif.) specifications. The average coefficient of variation for all LDH assays run in duplicate was less than 5%.

Stable carbon isotope analysis

Carbon isotope (^{13}C) analysis was performed on dried perch muscle tissue (dorsal, directly posterior to the head) packed in 4 mm × 6 mm tin capsules. ^{13}C, which is a naturally occurring heavy isotope of carbon, is useful for inferring feeding habits of fish because prey from different habitats of lakes (e.g., benthic vs. pelagic) typically have distinct ^{13}C signatures ($^{13}C/^{12}C$) and consumers acquire ^{13}C signatures similar to that of their prey (Vander Zanden et al. 1999). Stable carbon signatures were expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PeeDee belemnite limestone): $\delta^{13}C = ([R_{sample}/R_{standard}] - 1) \times 1000$; $R = {}^{13}C/{}^{12}C$). The average standard error for a subset of one-tenth of the samples run in duplicate was 0.09‰.

Bioenergetic analysis

Conversion efficiency ($K1$) was given as

$$(1) \qquad K1 = (G/C) \times 100$$

where G is specific growth rate ($g \cdot g^{-1} \cdot day^{-1}$) and C is specific consumption rate ($g \cdot g^{-1} \cdot day^{-1}$). G can be estimated from the following bioenergetic model (Kitchell et al. 1977):

$$(2) \qquad G = C - \mathrm{SMR} \times A - \mathrm{SDA} - F - U$$

where SMR is the standard metabolic rate, A is fish activity, SDA is specific dynamic action, F is egestion, and U is excretion. In traditional formulations of this model, both C and A are unknowns (although changes in activity are usually assumed to be negligible) and SMR, SDA, F, and U are generic parameters derived from laboratory experiments; SMR is a function of body size and water temperature, and SDA, F, and U are fixed proportions of C (totalling about 40%) (Kitchell et al. 1977). For our purposes, C was assumed (4% body weight per day for all lakes and body sizes); no systematic difference exists in consumption rates of perch among lakes of varying metal contamination (Sherwood et al. 2000), and C is isometric in yellow perch (Sherwood et al. 2002). Fish activity was included as a function of LDH activity (Fig. 1), which scales positively to body size and is dependent on diet on a lake-specific basis (Fig. 2).

Statistics

For perch from the Eastern Townships, published fish activity values (Boisclair and Leggett 1989) were regressed against measured LDH activity values (log transformed). Fish activity values were for age-2+ and age-3+ perch from five different lakes (Brome, Bromont, Magog, Memphremagog, and Waterloo; total $n = 9$; data for Brome age-2+ perch was lacking). LDH values were from regression of LDH versus body size in these five lakes. For Abitibi perch, size- and diet-related changes in LDH activity were described by the following regression model:

$$(3) \qquad \log \mathrm{LDH} = a \cdot \log W + b - c - d$$

where W is body size, a is slope, b is intercept, and c and d are saw-toothed breaks that apply when fish shift diets, sequentially, to benthivory and piscivory (see Sherwood et al.

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(2002) for a comparison of diet-shift model with more traditional line-fitting techniques). The inclusion and positioning of saw-toothed breaks (i.e., parameters *c* and *d*) was aided from both diet and $\delta^{13}C$ observations. Saw-toothed breaks were not included when diet shifts were not well defined as in the case of the two metal-polluted lakes where simple linear regression was employed. Area of diet overlap (region of shift, Fig. 2) was not included in regression analysis (eq. 3) for reference perch.

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Niche segregation between Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*): an experimental study of mechanisms

Peder A. Jansen, Henning Slettvold, Anders G. Finstad, and Arnfinn Langeland

Abstract: Interactive competition has been suggested to be an important mechanism by which brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) segregate into benthic vs. pelagic niches. According to the interactive competition hypotheses, Arctic char and brown trout should have the same preference for prey. We tested this by studying foraging performance when char and trout were offered small pelagic *Daphnia longispina* and (or) large epibenthic *Gammarus lacustris* in 10-min foraging experiments with solitary fish and with fish competing pairwise. There were obvious behavioural differences between char and trout. Trout were profoundly more aggressive than char. In comparison, char chose small pelagic daphnids and were superior daphnid foragers. Trout chose large epibenthic gammarids and were superior gammarid foragers. When competing, char and trout segregated such that rate of feeding on the chosen prey type was similar to solitary foraging fish, whereas rate of feeding on the alternative prey type was close to zero. We suggest that the observed selective differences in foraging behaviour, choice of prey, and feeding rates play an important role in niche segregation between Arctic char and brown trout. Hence, our results conform more closely with selective processes, rather than interactive processes, as the founding mechanisms for such segregation.

Résumé : On croit que la compétition interactive est un mécanisme important qui amène la Truite brune (*Salmo trutta*) et l'Omble chevalier (*Salvelinus alpinus*) à s'isoler respectivement dans une niche benthique et une niche pélagique. D'après les hypothèses reliées à la compétition interactive, l'Omble chevalier et la Truite brune devraient avoir une préférence pour les mêmes proies. Nous avons évalué cette préférence en étudiant la performance de la quête de nourriture chez des ombles et des truites isolées et en compétition deux par deux; dans des expériences de quête de nourriture pendant 10 min, nous leur offrions de petites proies pélagiques, *Daphnia longispina*, et (ou) de grandes proies épi-benthiques, *Gammarus lacustris*. Il y avait des différences de comportement évidentes entre l'omble et la truite. Les truites étaient beaucoup plus agressives que les ombles. Par comparaison, les ombles choisissaient les petites daphnies pélagiques et étaient des consommateurs plus efficaces de daphnies. Les truites préféraient les gros gammares épibenthiques et étaient de meilleurs prédateurs de gammares. Dans les expériences de compétition, les ombles et les truites se séparaient de telle façon que les taux d'alimentation et le choix des proies étaient les mêmes que chez les poissons isolés; le taux d'alimentation sur les proies de rechange était à peu près nul. Nous croyons que les différences sélectives dans le comportement alimentaire, le choix des proies et les taux d'alimentation que nous avons observés jouent un rôle important dans la ségrégation des niches chez l'Omble chevalier et la Truite brune. En conséquence, nos résultats s'accordent mieux avec l'hypothèse basée sur les processus de sélection qu'avec celle reliée aux processus interactifs comme mécanisme explicatif d'une telle ségrégation.

[Traduit par la Rédaction]

Introduction

Segregation by habitat is a common means by which ecologically similar species partition resources (Schoener 1974). Studies of closely related and ecologically similar species pairs of freshwater fishes have documented segregation into benthic vs. pelagic niches when living in sympatry; green sunfish (*Lepomis cyanellus*) and bluegill sunfish (*Lepomis macrochirus*) (Werner and Hall 1976, 1977), cutthrout trout (*Oncorhynchus clarki*) and Dolly Varden char (*Salvelinus malma*) (Andrusak and Northcote 1971; Hindar et al. 1988), as well as brown trout (*Salmo trutta* L.) and Arctic char (*Salvelinus alpinus* (L.)) (Nilsson 1967; Svärdson 1976; Langeland et al. 1991).

A pattern emerging from these studies is an asymmetric effect of one species on the other. Nilsson (1963, 1965) ar-

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P.A. Jansen,[1] H. Slettvold, A.G. Finstad,[2] and A. Langeland.[3] Department of Zoology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway.

[1]Present address: Norwegian Ministry of Fisheries, P.O. Box 8118 Dep, N-0032 Oslo, Norway.
[2]Present address: Department of Natural History, Norwegian University of Science and Technology, N-7491 Trondheim, Norway.
[3]Corresponding author (e-mail: Arnfinn.Langeland@chembio.ntnu.no).

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gued that resource utilization of brown trout and Arctic char is similar in allopatry, and that both species prefer relatively large epibenthic or surface insect prey found most abundantly in littoral areas. In sympatry, the more territorial and aggressive trout displace char from littoral areas to pelagic or deeper epibenthic habitats (Nilsson 1963, 1965). Hence, Nilsson emphasized interactive competition between the species causing niche segregation. In parallel, Hindar et al. (1988) and Andrew et al. (1992) noted that experimental populations of allopatric Dolly Varden char increased their use of shallow-dwelling zoobenthos, and expanded habitat distribution compared to that in sympatry. Allopatric cutthroat trout, however, showed little change in proportions of major prey types used and in habitat distribution. The authors argue that aggressive dominance of cutthroat trout is likely to explain the greater effect of competition on Dolly Varden char. Werner and Hall (1976, 1977) studied the effect of competition for preferred prey between the congeneric bluegill and green sunfishes. The authors showed a strong asymmetry in the competition function favouring the more aggressive green sunfish in the most prosperous vegetation habitat.

Arctic char and brown trout are well suited for studies of competitive interactions since they co-exist over extensive time in relatively simple communities and their morphologies are similar, as are their habitat and resource use in allopatric populations (Nilsson 1963, 1965). A remarkable diversity of dominance ratios and flexibility in population size structures exist among sympatric char and trout populations, and there is a long history of studies on competitive interactions between natural populations of these species in Scandinavia (see Svärdson 1976). Such studies have mainly relied on comparisons of habitat and resource utilization between allopatric and sympatric populations (Nilsson 1967; Langeland et al. 1991). However, different resource levels between study sites may confound comparative studies of habitat and resource use (Werner 1986).

Against this background, we conducted experiments to study foraging performance in juvenile (1+ years old) Arctic char and brown trout preying on small pelagic *Daphnia longispina* O.F.M. and large benthic *Gammarus lacustris* G.O. Sars, and relate such performance to interactive vs. selective segregation. Specifically, we tested the prediction based on Nilsson (1963) that both char and trout should prefer the relatively large gammarids to small daphnids when feeding without interference from competitors. Furthermore, we compared rates of feeding on small daphnids and large gammarids between the species by offering each prey singly to solitary feeding char and trout. Finally, we studied the effect of interactive competition on feeding rates by letting pairs of char and trout compete for both prey types simultaneously, or for either prey singly. Agonistic behaviour of the fish during experiments was also recorded.

Materials and methods

Predators and prey

All fish used in the experiments were first-generation hatchery-reared offspring. The brown trout originated from the alpine Lake Femunden and the Arctic char from the alpine Korssjøen, both in Central Norway. Lake Femunden holds a multispecies community of fish. Korssjøen holds a monomorph zooplankton-consuming Arctic char population along with sympatric brown trout. The fish had been fed only standard aquaculture fish feed prior to experiments. Fish were conditioned to experimental tanks and prey at least one week prior to experiments. During the conditioning and experimental periods, fish were fed only experimental prey. Prey were regularly collected from natural ponds and stored in the laboratory.

Experimental procedures and treatments

Experimental tanks were 120-L glass aquaria (80 cm × 40 cm × 40 cm) filled with 100 L of water. The aquaria were divided into two compartments (25 and 75 L) by a transparent gate. The bottom substrate consisted of pebble (1–5 cm in diameter, off-white to brown in colour). Water temperatures were kept at 11–13°C with continuous water flow through the aquaria (0.5 $L \cdot min^{-1}$). Untreated water from Jonsvatnet, Trondheim, was used. Light intensity in daylight varied (underwater quantum sensor LI-192SA, LI-COR, inc., Lincoln, Nebr., U.S.A.) in the range 2.8–4.2 × 10^{17} photons$\cdot m^{-2} \cdot s^{-1}$ (5.4–8.2 lx), in a 16 h day : 8 h night cycle.

All experimental aquaria were stocked with six fish; either three trout and three char (mixed species assemblage), or six trout or six char (conspecific assemblages). The fish were size differentiated for individual identification so that two were small (size range: 71–100 mm for trout; 74–107 mm for char), two were medium sized (84–121 mm for trout; 81–142 mm for char), and two were large (90–152 mm for trout; 109–161 mm for char). Prior to experiments, all fish were shut into the small aquarium compartment. One of three prey-type assemblages was added to the large compartment: (*i*) one-hundred *D. longispina* (counted using a pipette) of sizes 1.8 mm (±0.3 mm standard deviation (SD), $N = 123$), (*ii*) seven *G. lacustris* of sizes 7.4 mm (±0.88 SD, $N = 30$), or (*iii*) two prey types (100 daphnids + 7 gammarids). Daphnids were allowed to distribute and gammarids were allowed to settle on the bottom substrate for 3 min.

Experiment 1: solitary foraging fish

To test for difference in rate of feeding on the two prey types, solitary feeding fish were offered both prey types simultaneously, or either prey type singly. Solitary fish were shunted through the gate, or netted, to the large compartment containing prey. During the following 10 min the number of each prey type consumed was counted by eyesight. After experiments, fish were netted back to the small compartment. Excess prey were removed before subsequent experiments.

The experimental procedure was followed in a successive series for all six fish in experimental fish assemblages. In aquaria with conspecific fish assemblages, similar-sized pairs of fish were tested subsequently ensuring one test for all fish in a series of experiments. Otherwise, the sequence of fish tested was random. On rare occasions, when individual fish apparently did not actively seek prey, the results were discarded. Hence, we at least duplicated, and sometimes triplicated, every successive series of experiments. From this data we used the mean number of prey consumed during 10-min experiments for individual fish in later analyses. This procedure produced independent rates of feeding on the offered prey types for all fish in an assemblage (Table 1). Fish from

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both mixed and conspecific fish assemblages were tested. Fish assemblages were only used for one prey-type assemblage.

Experiment 2: two fish competing

To test whether competition alters feeding rate compared to solitary foraging fish, pairs of trout and char were offered prey. The competition experiments were conducted upon conclusion of the solitary feeding experiments, with the same fish assemblages being offered the same prey assemblages. Competition experiments were only done in mixed-species fish assemblages. The number of gammarids consumed during 10-min experiments was counted for both fish simultaneously. The number of daphnids consumed in 30 s was counted for one randomly chosen fish and then the number was counted for the competitor fish the next 30 s, throughout experiments. After experiments, fish were netted back to the small compartment. Excess prey were removed before subsequent experiments.

In a series of competition experiments within a fish assemblage each trout competed pairwise against each char, producing nine experimental trials. The sequence of trials with individual fish was random. Feeding rate for individual fish was calculated from the mean number of prey consumed in three experimental trials (against each of the opponent species). No experiments were discarded. This procedure produced rates of feeding on the offered prey types for all six fish in each of the experimental fish assemblages (Table 1).

Prey and predator behaviour

To relate prey consumption to fish behaviour, we present descriptive observations on prey and predator behaviour. In the competition experiments, we recorded the number of attacks launched by one fish towards the other. An attack was recorded when one fish chased the other with accompanying threat display (Kalleberg 1958). We also noted when gammarids were caught on the substratum or swimming in the water column.

Data analyses

The rate of feeding on prey is presented as the mean number of prey consumed per fish over the 10-min experimental period. In the competition experiments we doubled the observed number of daphnids consumed as an estimate of the total number consumed, since daphnid consumption was only observed every half minute per fish. Furthermore, rate of feeding on prey and the number of launched attacks for individual fish is averaged over the three experimental trials, involving each fish from the opponent species group. All statistical tests used are Student's t tests (two tailed). The threshold level of significance was set at $p < 0.05$.

Results

Prey and predator behaviour

The gammarids distributed in a plane over the substrate where they could be hidden when inactive. When active, the gammarids crawled over the substratum, or swam, mostly over short distances and close to the substratum. The daphnids distributed over the volume of the tank.

Trout searching for gammarids normally positioned centrally in the aquarium, with the tail fin close to the substratum. When gammarids were encountered, quick, voracious attacks were launched, after which the fish returned to the fixed position. Trout caught 54% of gammarids on the substratum and 46% swimming in the water column ($n = 626$). Daphnid consumption by trout was characterized by an uneven start and stop movement.

Char typically cruised about the aquarium. Attacks on gammarids were slower and more hesitant than for trout. Char caught 11% of gammarids on the substratum and 89% in the water column ($n = 218$). Char normally picked consecutive daphnids while cruising at a constant speed, and were consistently positioned higher in the water column than trout.

Trout were markedly more aggressive than char (Figs. 1*a*–1*c*). Prey assemblage did not significantly affect the number of launched attacks for either char or trout. Analyses of relationships between the number of launched attacks and prey consumption did not give conclusive results.

Experiment 1: solitary foraging fish

There was no significant effect of mixed vs. conspecific fish assemblage on feeding rate in any of the three prey-type assemblages (Figs. 2*a*–2*d*), with the exception of trout feeding on gammarids in one prey-type experiment ($t_{13} = 2.25$, $p = 0.042$). Hence, results from mixed and conspecific assemblages are pooled in further statistical treatment.

Char and trout clearly chose different prey types. Char consumed nine times the number of daphnids consumed by trout, whereas trout consumed 3.2 times the number of gammarids consumed by char (Figs. 2*a*, 2*c*). No fish diverged from this choice of prey-type pattern since all char consumed more daphnids than any trout, and vice versa concerning gammarids.

Char and trout choice of prey type was related to differences in consumption rate. Char feeding rate was significantly higher than for trout when offered only daphnids ($t_{22} = 3.31$, $p = 0.003$; Fig. 2*b*). Conversely, trout feeding rate was significantly higher than for char when offered only gammarids ($t_{28} = 5.63$, $p < 0.001$; Fig. 2*d*).

Prey assemblage significantly influenced char and trout rate of feeding on the nonchosen prey, but not that of the chosen prey. Char more than doubled rate of feeding on gammarids when offered only such, compared with when two prey types were offered ($t_{43} = 6.27$, $p < 0.001$; Figs. 2*c*, 2*d*). Conversely, trout increased rate of feeding on daphnids nearly sevenfold when offered only such, compared with when two prey types were offered ($t_{40} = 9.15$, $p < 0.001$; Figs. 2*a*, 2*b*). Rate of feeding on daphnids for char was 66.9 and 65.8 (Figs. 2*a*, 2*b*), and rate of feeding on gammarids for trout was 4.93 and 4.90 (Figs. 2*c*, 2*d*), in experiments with one prey type and two prey types, respectively.

Experiment 2: two fish competing

When char and trout competed for two prey types, they segregated. Char rate of feeding on gammarids was only 0.3, whereas rate of feeding on daphnids was similar to solitary foraging char (Figs. 3*a*, 3*c*). Trout feeding rate on daphnids was less than 5, whereas rate of feeding on gammarids was similar to solitary foraging trout (Figs. 3*a*, 3*c*).

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Table 1. Number of aquaria and fish used in the foraging experiments with different prey and fish assemblages.

Prey assemblage	Fish assemblage	No. aquaria	No. fish	
			Arctic char	Brown trout
Two choice	M	6	18	18
	C	4	12	12
Single-prey daphnids	M	2	6	6
	C	2	6	6
Single-prey gammarids	M	3	9	9
	C	2	6	6

Note: C, conspecific; M, mixed species.

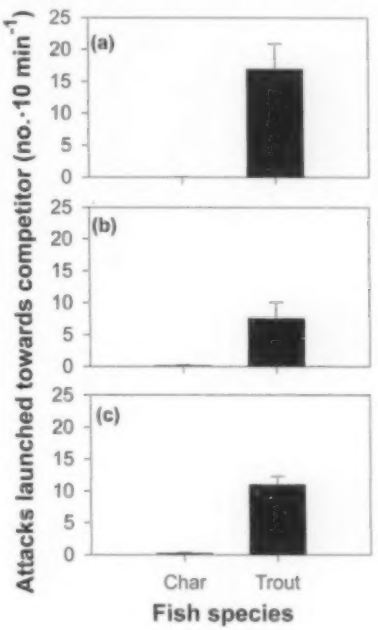
The effect of trout on rate of char feeding on the nonchosen prey was more severe than the effect of char on feeding rate of trout on the nonchosen prey. Trout rate of feeding on gammarids was about 15-fold that of char in trout–char competition for one prey type (Fig. 3*d*). Conversely, char rate of feeding on daphnids was about double that of trout (Fig. 3*b*).

Discussion

The present foraging experiments revealed marked differences between char and trout in behaviour, choice of prey type and rate of feeding on the two prey types. Trout aggression and territoriality agree fully with previous reports (Kalleberg 1958; Nilsson 1963). Char, on the contrary, showed little aggression, positioned high in the water column, and cruised constantly during foraging. Comparatively, char chose small pelagic *D. longispina*, whereas trout chose large epibenthic *G. lacustris*, when given the option to prey on both prey types. Feeding rate varied significantly between the species according to choice of prey type when only one prey type was offered. When offered both prey types, the presence of nonchosen prey did not affect rate of feeding on chosen prey. The fish used had only experienced standard aquaculture fish feed prior to experiments. Thus, prey choice and consumption rate reflected innate differences between experimental Arctic char and brown trout, and was not influenced by previous learning.

The differences in choice of prey type, feeding rate, and behavioural performance emphasize a selective foundation for niche segregation between Arctic char and brown trout. Our prediction, based on Nilsson (1963), that both species would prefer gammarid prey, was rejected. Nilsson's (1963) basis for suggesting similar prey preference in char and trout was partly founded on foraging experiments and partly founded on observing similar diets in stomach contents from allopatric char and trout. In the foraging experiments food pellets of different coloration were offered to char and trout, and either on the surface or on the bottom of aquaria. Although behavioural differences were emphasized, there was no apparent difference in the ranking of food categories between char and trout. Nilsson concluded that trout and char had the same preferences for the offered foods. This discrepancy compared to our results may relate to the different experimental foods. It is more likely that the natural prey offered in the present study more accurately reflects prey

Fig. 1. Mean (+ standard error) number of launched attacks over 10 min towards competitor in Arctic char and brown trout pairwise competition experiments with (*a*) single prey type daphnia, (*b*) single prey type gammarus, and (*c*) two prey types.



choice in nature. Furthermore, no pelagic prey was offered in Nilsson's (1963) experiments.

Nilsson (1963, 1965) reported striking similarities in resource utilization between natural populations of allopatric char and trout. Both species were reported to prefer *Gammarus*, *Lymnaea*, ephemerid nymphs, and terrestrial insects (Nilsson 1963). These are relatively large prey found abundantly in littoral and near-shore areas. However, there are at least two problems with Nilsson's conclusions. First, there is no mention of the size of char and trout that Nilsson studied other than size distributions being similar between species. Judging from the composition of mesh sizes in the gang of gillnets used to sample fish (Nilsson 1965), and more recent studies on gillnet selectivity (e.g., Finstad et al. 2000), it is probable that the sampled fish have been grossly biased towards the larger fish in the populations. Effects of size on trophic ecology (e.g., size-related foraging efficiency and energy demand reinforcing ontogenetic niche shifts in fish) have increasingly been emphasized (see Werner 1986; Ebenman and Persson 1988). Arctic char are commonly reported to shift from foraging small prey in the pelagic to larger benthic prey (Hindar and Jonsson 1982; Forseth et al. 1994). Thus, we suspect that pelagic zooplankton prey plays a more dominant role as a resource for allopatric char than was inferred form Nilsson's samples, as is supported by more recent studies (Langeland 1986; Langeland et al. 1991). Further contributing to this line of evidence is that zooplanktivorous char may do very well in terms of growth and food acquisition (Hegge et al. 1989; P.A. Jansen, personal observations). Second, niche comparisons between allopatric and sympatric populations are likely to be con-

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Fig. 2. Mean (+ standard error) rate of feeding on (*a*, *b*) daphnids and (*c*, *d*) gammarids by solitary foraging fish offered both daphnids and gammarids (two prey types, upper panels), or either prey type singly (single prey type, lower panels), over 10-min experiments. Solid bars, conspecific experimental units; open bars, mixed species units.

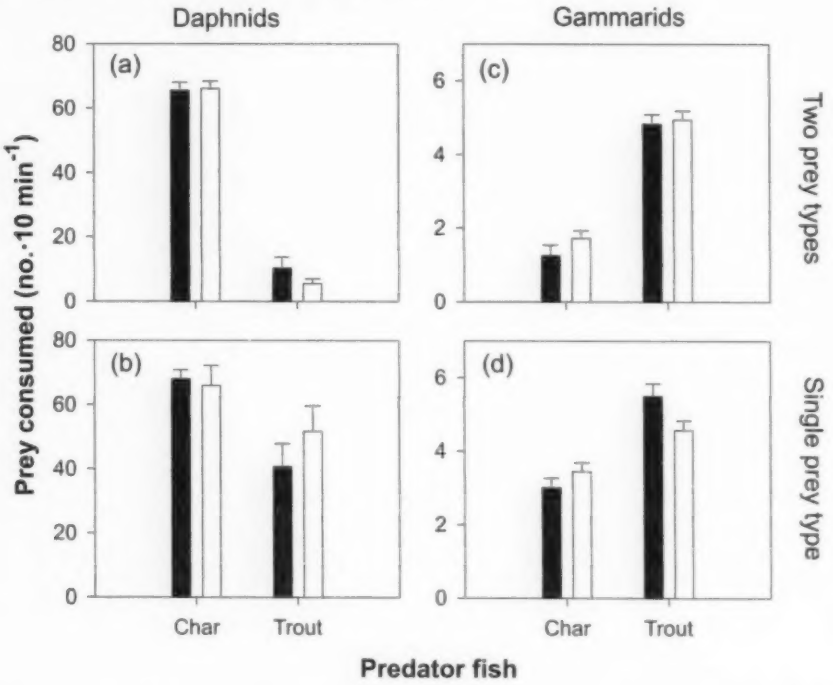
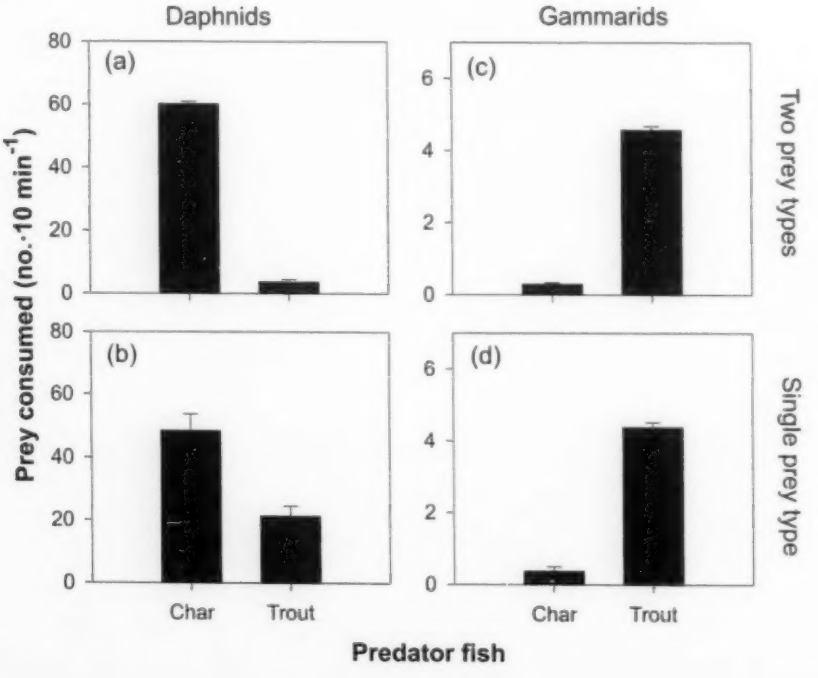


Fig. 3. Mean (+ standard error) rate of feeding on (*a*, *b*) daphnids and (*c*, *d*) gammarids by pairwise-competing Arctic char and brown trout offered both daphnids and gammarids (two prey types, upper panels), or either prey type singly (single prey type, lower panels), over 10-min experiments.



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founded by differences in resource abundance or availability between study sites (Nilsson and Pejler 1973; Werner 1986).

In the competition experiments described here, char and trout segregated with respect to proportions of daphnids and gammarids consumed when offered both prey types. Consumption of the nonchosen prey was close to zero for both species. This was a trivial result since prey depletion during the experiments was in the order of 60–80% for both prey types, which implied that after having depleted the chosen prey type, also the alternative prey type would be depleted by the competitor fish. Nevertheless, the result is instructive in demonstrating that exploitation of resources can lead to interactive segregation (sensu Nilsson 1967).

Apart from trout being profoundly more aggressive than char, there were no simple relationships between aggressive behaviour and prey consumption. However, aggressive behaviour may be more beneficial in holding favourable foraging positions in the long run, as shown for stream salmonids (e.g., Nakano et al. 1999), than in the present experiments where prey abundance quickly diminished. Nevertheless, the profound aggression in trout illustrates a potential for interference processes playing a role in segregation between Arctic char and brown trout. Also, the present results agreed with the notion that trout had a larger competitive effect on char than vice versa (Nilsson 1967; Hindar et al. 1988; Langeland et al. 1991).

In conclusion, the present foraging experiments revealed selective differences between small Arctic char and brown trout in foraging behaviour, choice of prey, and rate of feeding on prey. In comparison of the species, Arctic char chose small pelagic daphnids and were superior daphnid foragers with respect to feeding rate. Brown trout chose large epibenthic gammarids and were superior gammarid foragers. We suggest that these selective differences in foraging performance play an important role in niche segregation between Arctic char and brown trout. Hence, our results conform more closely with selective processes, rather than interactive processes, as the founding mechanisms for such segregation. This conclusion was also reached by Schutz and Northcote (1972) in an experimental study of feeding behaviour and interaction of cutthroat trout and Dolly Varden char.

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The influence of hydrological and biotic processes on brown trout (*Salmo trutta*) population dynamics

Franck Cattanéo, Nicolas Lamouroux, Pascal Breil, and Hervé Capra

Abstract: Hydrological and biotic forces constrain brown trout (*Salmo trutta*) population dynamics, but tests of their role across numerous streams are uncommon. In 30 French stream reaches, using 5–8 samples (1 per year) each, we investigated whether the year-to-year seasonal hydrology influenced annual trout densities within reaches, and whether the relationships were shared by all reaches. We also searched for intraspecific interactions between and within cohorts. Trout data were age class (0+, 1+, and adults) densities. For each year, hydrology was described using 13 variables, each computed for a reproduction, emergence, and growth period related to the biological cycle of trout. We used analyses of covariance (ANCOVA) to test how trout densities at year $n - 1$ and hydrology at year n influenced trout densities at year n. High flows during emergence significantly reduced the 0+ densities, consistently across the 30 reaches. Then, 1+ and adult densities were linked, respectively, to 0+ and 1+ densities from the previous year. Analyses also revealed density-dependent survival mechanisms for the 0+ cohort, suggesting intracohort competition. Therefore, hydrology constrains trout dynamics only during the critical emergence period, after which intracohort interactions regulate the 0+ density. Such mechanisms, validated across 30 environmentally different reaches, seem to be fundamental to trout population dynamics.

Résumé : Les forces hydrologiques et biotiques imposent des contraintes à la dynamique de population des Truites brunes; cependant, on a rarement évalué leur rôle simultanément dans un grand nombre de cours d'eau. À partir de 5–8 échantillons (1 par année) récoltés dans chacun de 30 tronçons de cours d'eau français, nous avons examiné si les variations de l'hydrologie d'année en année influencent la densité annuelle des truites dans un tronçon et si les relations obtenues s'appliquent à tous les tronçons. Nous avons aussi cherché à trouver des interactions intraspécifiques au sein des cohortes et d'une cohorte à l'autre. Nos données consistaient en des densités des différentes classes d'âges (0+, 1+ et adulte). Nous avons mesuré, chaque année, 13 variables hydrologiques, regroupées selon les périodes d'émergence, de reproduction et de croissance du cycle biologique des truites. Des analyses de covariance (ANCOVA) nous ont permis de vérifier comment la densité des truites de l'année n – 1 et l'hydrologie de l'année n pouvaient influencer la densité des truites de l'année n. Des débits élevés durant l'émergence réduisaient significativement les densités des truites d'âge 0+, de façon comparable dans tous les 30 tronçons. De plus, les densités des truites d'âges 1+ et adulte étaient reliées respectivement aux densités des poissons d'âges 0+ et 1+ de l'année précédente. Les analyses ont aussi mis en évidence des mécanismes de survie reliés à la densité chez la cohorte d'âge 0+, ce qui semble indiquer l'existence de compétition au sein de la cohorte. L'hydrologie impose donc des contraintes à la dynamique de population des truites seulement durant la période critique de l'émergence, après quoi ce sont les interactions au sein de la cohorte qui contrôlent la densité des poissons d'âge 0+. De tels mécanismes, observés dans 30 tronçons qui diffèrent par leurs conditions environnementales, semblent être des caractéristiques fondamentales de la dynamique de population des truites.

[Traduit par la Rédaction]

Introduction

Salmonid populations exhibit large variations in population size, in both space and time. The spatial variability is mainly affected by large- to local-scale environmental factors (e.g., climate, topography, local habitat features) that act as filters on fish occurrence, abundance, or production (Poff 1997). Through time, salmonid population dynamics are driven by a combination of both density-dependent and density-independent processes, whose respective influence

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F. Cattanéo.[1] Cemagref, U.R. Biologie des Ecosystèmes Aquatiques, Laboratoire d'Hydroécologie Quantitative, 3 bis quai Chauveau, CP220, 69336 Lyon CEDEX 09, France, and Cemagref, U.R. Hydrologie-Hydraulique, 3 bis quai Chauveau, CP220, 69336 Lyon CEDEX 09, France.
N. Lamouroux and H. Capra. Cemagref, U.R. Biologie des Ecosystèmes Aquatiques, Laboratoire d'Hydroécologie Quantitative, 3 bis quai Chauveau, CP220, 69336 Lyon CEDEX 09, France.
P. Breil. Cemagref, U.R. Hydrologie-Hydraulique, 3 bis quai Chauveau, CP220, 69336 Lyon CEDEX 09, France.

[1]Corresponding author (e-mail: cattaneo@lyon.cemagref.fr).

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depends mainly on the life stage of the fish and the environmental conditions (Elliott 1989). Amongst these factors, hydrological variability, food availability, and biological interactions between individuals have been demonstrated to be of major importance (Elliott 1989; Nehring and Anderson 1993; Jowett 1995).

Studies that have previously attempted to assess the influence of hydrological patterns on fish population dynamics have often involved one or a few sites, within a single stream or a few geographically close streams, although long-term investigations have sometimes compensated for this limitation (Nelson 1986; Elliott 1989; Cattanéo et al. 2001). In a review of models derived to predict fish standing crop, Fausch et al. (1988) indicated that most models transferred poorly to other streams, and therefore questioned their ecological relevance. A means of strengthening the ecological significance of a relationship is to test for its consistency across several distinct sites and years. The more a relationship is consistent across a wide diversity of sites, the higher the chance for this relationship to be ecologically relevant. At present, substantial validations of the influence of hydrology on fish population dynamics across multiple sites and years are scarce (e.g., Nehring and Anderson 1993).

Some authors have investigated simultaneously the influence of flow variability and biotic interactions on salmonid population dynamics. In six Colorado streams, Latterell et al. (1998) found that 1+ trout densities were negatively affected by the 30-day maximum flow and adult stock the previous year. Their model was relatively precise (R^2 = 73%), but neither the variability explained by flow variables nor that explained by adult trout densities was described. It is now widely recognized that the impact of hydrological events does not depend only on their magnitude, but also on their timing relative to the fish developmental stage affected. In Black Brows Beck, Elliott et al. (1997) estimated parameters of a Ricker stock–recruitment model for different life stages of a sea trout (*Salmo trutta*) population and demonstrated that outliers of this model generally corresponded with years that experienced a summer drought. The 0+ and 1+ stages were the more sensitive and were negatively affected by summer droughts, possibly by reduction of available suitable habitat. More generally, most investigations concluded that the early 0+ stage was particularly sensitive to hydrological variability, owing to the inability of 0+ fish to cope with large environmental fluctuations (i.e., inability to find shelters or to face high current velocities). This can lead to bottlenecks in the population dynamics. For example, Nehring and Anderson (1993) reported a detrimental effect of high spring flows on emerging trout fry in 11 Colorado streams, as did Jensen and Johnsen (1999) in two Norwegian rivers. In the Chena River (Alaska), Clark (1992) attempted to relate year class strengths of arctic grayling (*Thymallus arcticus*) with spawner abundances and the flow regime during the spawning, emergence, and the larval stage. He failed to demonstrate any stock–recruitment relationship, but showed that high discharges from the spawning to larval stage had a negative impact on recruitment three years after. Therefore, long-term fish population dynamics can markedly display the influence of hydrological events. An extreme case was reported by Beaudou et al. (1995) on a Corsican river that experienced a 40-year flood. The trout population almost entirely disappeared and only few individuals found shelter in less affected upstream tributaries. However, two years were sufficient to recover a normal size-structured population.

Relationships between cohorts at any given time can also affect age class densities or survival. Although stream salmonids have distinct habitat requirements depending on their size (i.e., age class), habitat use overlap can occur. For example, biotic interactions can result from ontogenic shifts in habitat used. Symons and Héland (1978) reported interactions between 1+ (>10 cm) and 0+ (<6 cm) Atlantic salmon (*Salmo salar*). Once the 0+ fish reached 6.5 cm, their preference for deeper habitats increased and social interactions with 1+ fish were displayed by way of territorial defence. Intercohort interactions between the 0+ and 1+ fish have also been demonstrated for brown trout (Bohlin 1977).

In this paper, using fish and hydrological data collected over 5–8 years in 30 scattered French reaches, we analyzed simultaneously how trout populations, described by age class densities at year n, depended on both the year-to-year seasonal hydrology and age class trout densities at year $n - 1$. We did not focus on variations in age class densities between reaches, which could be affected by factors other than hydrology (e.g., nutrient availability, temperature, habitat complexity). Instead, our major goal was to test whether within-reach changes in trout densities could be explained by a biological or hydrological factor, and whether the different reaches statistically shared such relationships. Finally, we discuss the relative role of hydrology and biotic processes for trout population dynamics.

Material and methods

Study sites

This study involved 30 stream reaches (179 reaches × years combinations) for which both fish and hydrological data were available (Fig. 1). Fish data came from a national database held by the Conseil Supérieur de la Pêche, grouping about 800 stream reaches sampled annually across France. Hydrological data (daily discharges, Q_d) were extracted from the Hydro national database, held by the French Department of the Environment, and included approximately 3500 gauged sites. The 30 study reaches were selected from the fish database using the following criteria: (*i*) the fish sampling reaches had to be close to a gauging station located on the same river (in our case, the distance between the fish and hydrological sites ranged from 0.3 to 16.6 km, with a median distance of 6.6 km); (*ii*) the average proportion of brown trout vs. other species in a reach had to be greater than 0.25; (*iii*) reaches had to be sampled for at least five continuous years; (*iv*) the daily discharge series had to include the fish sampling period; and (*v*) each year, reaches had to be sampled during the low flow period, in summer or early autumn (to ensure a suitable catchability of the 0+ fish). For a given reach, the sampling was carried out at a comparable date in the year, and at least three months after emergence, to minimize bias because of the sampling period. The selected reaches were widespread across France, and encompassed a large diversity of environmental conditions (mountain to lowland sites and oceanic-influenced to Mediterranean-influenced reaches). Reaches were between 1.2 m

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Fig. 1. Location of the 30 study reaches in France.

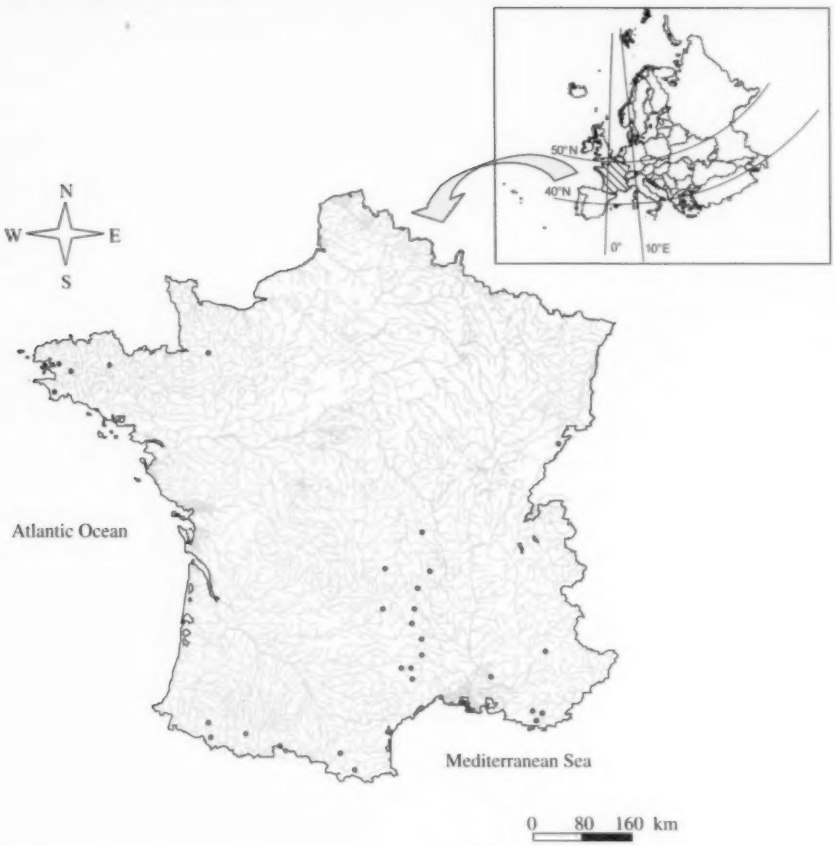

Table 1. Physical characteristics of the study reaches.

Physical characteristics	*N*	Min.	Mean	Max.
Width (m)	30	1.2	7.5	20.5
Reach slope (%)	28	0.1	1.2	6
Elevation (m)	30	15	552	1320
Distance from the source (km)	30	1	18.1	75
Basin area (km^2)	30	1	103.1	450
Median discharge ($m^3 \cdot s^{-1}$)	30	0.02	3.9	34.8
% of run	19	25	61.6	100
% of riffle	19	0	28.7	65
% of pool	19	0	9.7	40

Note: *N*, number of reaches with data. Values: Min., minimum; Mean, mean; Max., maximum. Except for reach slope, elevation, distance from the source, and basin area, the given characteristics are average values computed on all samples in a reach.

and 20.5 m wide; they were located from 1 to 75 km from the source, at altitudes ranging from 15 to 1320 m, with a reach stream slope between 0.1% and 6%, a drainage area between 1 km^2 and 450 km^2, and an interannual median discharge from 0.02 to 34.8 $m^3 \cdot s^{-1}$ (Table 1). Among these 30 reaches, five were subject to artificial hydrological regimes: four had water extraction resulting in minimum flow regimes and one was subject to hydropeaking.

Annual fish data

Fish were sampled by two-pass removal electrofishing. The two-pass method gave reliable estimates of fish abundance: about 80% (standard deviation, SD = 15.3%) of fish were caught during the first pass, thus demonstrating the global efficiency of this method. The sampling procedure involved 1 to 4 fishing teams (each of 3 to 4 persons), depending on the stream width, wading in an upstream direction. The fishing equipment was composed of a towed Héron-Dream Electronic 180–1000 V (1–4 A) direct current apparatus (assembled in Pessac, France) supplied by a Honda 5 kW (11 hp) generator. Prospected areas ranged from 135 to 3200 m^2, depending on the site, and included several pool–riffle sequences.

All fish were measured (total length) to the nearest millimetre. We defined three age classes after examination of length–frequency histograms. The 0+ fish (from emergence in year n to their first birthday in year $n + 1$) had a total length below 100 mm. The 1+ fish (from their first birthday in year n to their second birthday in year $n + 1$) had a maximum length of 179 mm. All fish with length >179 mm were grouped into a single "adult" category and were considered the potential reproductive pool.

Trout abundances were not always determined per unit pass, so we could not estimate the true density of fish. We

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therefore described trout populations at year n using cohort densities at year n, calculated as catch-per-unit-effort (i.e., the total number of trout caught after two passes standardized by the prospected area, expressed in number of fish after two passes·100 m^{-2}).

For each sample, age class densities were $\ln(x + 1)$ transformed to normalize their distribution. Because we looked for relationships between fish at year n and fish at year $n - 1$, we built a biological matrix with fish data in year n and year $n - 1$ on the same rows. We therefore "lost" one year for each reach. The final fish data matrix contained 149 samples in 30 reaches, with a minimum of four samples per reach. The biological characteristics of the 149 reaches × year combinations are recorded in Table 2.

Table 2. Biological characteristics of the 30 study reaches.

Biological characteristics	Min.	Mean	Max.
Total trout relative density (%)	25.6	72.1	100
Total trout density (fish·100 m^{-2})	6.9	32.9	100.3
0+ trout density (fish·100 m^{-2})	0.8	13.8	55.0
1+ trout density (fish·100 m^{-2})	1.8	13.4	48.2
Adult trout density (fish·100 m^{-2})	0.6	5.7	19.2

Note: Values are statistics based on average values by reach. For each characteristic, Min., minimum; Mean, mean; and Max., maximum average values of the 30 reaches.

Hydrological variables

From the national database, we extracted a 12-year series (from 1988 to 2000) of daily discharge data for the selected reaches. For each stream reach, we calculated the interannual median daily discharge ($Q50i$) computed over the entire data series.

The year-to-year hydrology was described for three seasons in the year, matching the main stages of brown trout annual biological cycle: (*i*) the "reproduction" period, starting on 1 October, continuing until 31 December (year $n - 1$); (*ii*) the "emergence" period, from 1 January until 31 May (year n); and (*iii*) the "growth" period, from 1 June until 31 August.

Season limits were the same in all sites, and were defined according to the literature. The spawning season starts in autumn and we can reasonably consider that most of the spawning activity occurs before January (Beard and Carline 1991; Crisp 1992). The emergence period included the emergence phase stricto sensu, the intragravel phase, and the larval and possibly the fry stages. Limits were chosen to fit with the dates reported in Crisp (1992) for three rivers with different temperature regimes. The growth period included the summer months. The end of the growth period was set on 31 August for all sites, which represented an "average" sampling date.

We defined 13 variables to describe the hydrological conditions at each reach × year × season (Table 3). Daily discharge series could contain some missing values, but less than 20% of values were missing for each reach × year × season combination. Variables reflected average conditions, low and high flow conditions, "infrequent" flow conditions, rate of change in daily discharge, and overall variability. Most variables were rescaled by the interannual median daily discharge $Q50i$ to reflect the magnitude of flow characteristics consistently in all reaches.

The average conditions of seasonal discharge were assessed using the median daily discharge divided by $Q50i$ (denoted $Q50$). We also computed the mean seasonal discharge divided by $Q50i$ (denoted QM), which could differ from the median discharge for regimes exhibiting skewness.

Low seasonal discharges were described by the discharge exceeded 90% of the time during the season, divided by $Q50i$ (denoted $Q90$). The minimum daily discharge value divided by $Q50i$, (denoted MIN) was also used.

High discharge values were described by three variables. The 10th percentile of the daily discharges distribution divided by $Q50i$ (denoted $Q10$) represented the discharge value exceeded 10% of the time over the season. The peak seasonal flow was assessed using the maximum daily discharge divided by $Q50i$ (denoted MAX). Because not only the magnitude but also the duration of a high flow event can be ecologically important, we computed the maximum discharge over a 7-day continuous period (Richter et al. 1996), divided by $Q50i$ (denoted MAX7).

We defined an infrequent high discharge threshold relative to each season. For each season, we estimated the statistical 2-year return period discharge, using the entire data series (1988–2000), by fitting an exponential law to discharge vs. empirical return period among high discharge values extracted from the data ("peaks over threshold" method, Lang et al. 1999). The choice of a 2-year return period discharge threshold enabled the hydrological data set to contain infrequent flows relative to the duration of the fish sample records for each site, which would have been scarce if we had chosen a higher threshold (5-year, for example). We then computed $NQ2$ as the number of times the seasonal discharge exceeded this threshold (i.e., if the threshold was exceeded several days on, only one event was counted) and $DQ2$ as the duration (in percentage of time during the season) above this threshold (see Cattanéo et al. 2001). Although some zero values appeared for $NQ2$ and $DQ2$ in the data matrix, this was not a restriction for further analyses. Similar variables were not computed in relation to a low flow threshold, since low flows are generally time-extended periods: such variables would have been redundant to $Q90$.

The rate of change in discharge can rapidly influence habitat availability and suitability and therefore limit fish abundances within a site. Thus, variations in discharge between two consecutive days were described. We first derived the series of daily discharge variations ($Q_d - Q_{d-1}$) over the season, and separated positive (i.e., increases) from negative (decreases) fluctuations. We then calculated the mean value for increases and decreases (Richter et al. 1996), both divided by $Q50i$ (denoted INC and DEC, respectively).

The overall seasonal variability was assessed using two descriptors. We first computed VAR, an index of variability based on the daily discharge fluctuations (Cattanéo et al. 2001). It was defined as the difference between the 10th and 90th percentile of the distribution of classified daily fluctuations, divided by $Q50i$. Finally, we calculated the coefficient of variation in daily discharges over the season (CV), defined as the ratio of the standard deviation of the daily discharge distribution divided by the mean.

For each season, flow variables were arranged in a reach × year × flow variables matrix (30 reaches in 149 rows × 13

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Table 3. Hydrological variables used and their definitions.

Flow characteristics	Variable	Definition
Average conditions	*Q*50	Median daily discharge divided by the interannual median discharge
	*Q*M	Mean daily discharge divided by the interannual median discharge
Low flows	MIN	Minimum daily discharge divided by the interannual median discharge
	*Q*90	Daily discharge exceeded 90% of time during the season divided by the interannual median discharge
High flows	MAX	Maximum daily discharge divided by the interannual median discharge
	MAX7	Maximum daily discharge over a 7-day period divided by the interannual median discharge
	*Q*10	Daily discharge exceeded 10% of time during the season divided by the interannual median discharge
Infrequent flows	*NQ*2	Number of times the daily discharge exceeds the 2-year high seasonal discharge (in number of events)
	*DQ*2	Percentage of time over the season where flow exceeds the 2-year high seasonal discharge
Rate of change	INC	Mean value for discharge increases between two consecutive days divided by the interannual median discharge
	DEC	Mean value for discharge decreases between two consecutive days divided by the interannual median discharge
Overall variability	VAR	Variability index, i.e., difference between the 10th and 90th percentiles of the distribution of discharge variations for two consecutive days divided by the interannual median discharge
	CV	Coefficient of variation in daily discharge, i.e., ratio of the standard deviation of the mean multiplied by 100

Note: All variables were computed by season. Most variables are dimensionless.

columns). Flow variables were $\ln(x + 1)$ transformed to normalize their distribution.

Data analysis

Within-reach variations in seasonal hydrology

Because our study focused on the within-reach variability in trout dynamics related to hydrological changes, we first analysed the within-reach hydrological variability (between years) for each season. For this purpose, we performed a within-reach principal components analysis (within-reach PCA, Dolédec and Chessel 1989) for each season. The within-reach PCA reduces the number of explanatory variables by defining independent synthetic variables (linear combinations of hydrological characteristics) that best reflect within-reach variations in hydrological patterns.

Which factors could explain changes in trout densities between years?

We first searched for interrelationships between cohorts at year n. Then, we related the annual age class densities at year n (0+, 1+, and adults) to age class densities at year $n - 1$ (0+, 1+, and adults) and hydrological axes during the biological year. All tests were made using covariance analyses (ANCOVA). The ANCOVA tests if there is a common effect within reaches of each explanatory variable on trout density at year n. In each ANCOVA performed, the reach was used as a group variable and each explanatory variable (biological or hydrological) used as a covariate. Models without covariates were used to test for between-reaches differences. ANCOVA is useful for comparing regression lines among groups (i.e., reaches), even if the dependent variable and the covariate differ in their mean values or ranges among groups (Sokal and Rohlf 1998). It is based on the model (model 1)

$$y = a_{\text{reach}} + b_{\text{reach}}\, x + e$$

where y was the biological dependent variable (trout densities at year n), a_{reach} an intercept depending on the reach, b_{reach} the slope parameter depending on the reach, x a hydrological or biological covariate (used as explanatory variable), and e a random deviation. If the interaction between the reach and the covariate was not significant (i.e., values for b did not differ across reaches), a common value for b was fitted for all reaches and we tested a simpler model (model 2):

$$y = a_{\text{reach}} + bx + e$$

The null hypotheses tested in model 2 were (*i*) the covariate x has no effect on the dependent variable, i.e., b was not statistically different from 0; and (*ii*) the dependent variable did not significantly differ between reaches, i.e., a was not statistically different between reaches.

The significant threshold for probabilities was corrected according to Bonferroni's method to provide protection for multiple tests (i.e., 30 tests). Therefore, probabilities were considered significant below 0.0017.

In the ANCOVA analyses, we used the synthetic hydrological variables (PCA axes) to avoid redundancy and multiplicity of tests. However, if there was a significant effect of a hydrological axis on a biological variable, we also tested a covariance model after replacing the PCA axis with a simpler hydrological variable representing this axis. This provided a better quantification of the effect of year-to-year hydrology on trout densities.

Was the 0+ fish survival density dependent?

If there was a significant relationship shared by all reaches between the 1+ fish and the 0+ fish the previous year, we attempted to investigate whether the survival function involved density-dependent mechanisms. For this purpose, we used the "unit-slope test" based on natural log (ln) transformed densities (Lebreton 1989). We included a con-

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stant to account for migration mechanisms; therefore, density dependence was assessed according to apparent survival rates. This test assumed that, under the hypothesis of no density dependence, the slope b of the covariance model relating the ln(densities) of a cohort at year n to the ln(densities) of the same cohort at year $n + 1$ is 1. Density dependence results in a slope significantly smaller than 1 (Lebreton 1989). However, this test suffers from inflated type I error in the presence of measurement uncertainties (i.e., concluding there is density dependence when there is none, Lebreton 1989). Therefore, we simulated virtual measurement errors on the 0+ and 1+ fish to check if they could influence our results.

Was 0+ fish survival affected by the hydrological conditions?

If there was a significant relationship shared by all reaches between the 1+ fish and the 0+ fish in the previous year, we checked whether the survival function was influenced by the hydrological conditions. To do this, we used an ANCOVA model as described above, with the 0+ fish density as first covariate, and successively added the hydrological variables (PCA axes) as second covariate. All possible interactions were tested and removed from the model in the case of nonsignificance. If the gain in explained variability owing to the second covariate was significant after Bonferroni's correction, we concluded that hydrology had an influence on the 0+ fish survival.

Results

Within-reach variations in seasonal hydrology

For each season, most of the within-reach variability could be projected onto the F1 × F2 factorial plane, which accounted for 71–84% of the total variance of hydrological variables within reaches (Fig. 2*a*). For each season, the first axis (53–65%) reflected the overall discharge level during the season (all variables were positively correlated with F1, except CV during the emergence period). The second axis (16–19%) reflected the shape of the hydrological signal, independently of the discharge level. The three variables CV, $NQ2$, and $DQ2$ had negative coordinates on F2, and were opposed to MIN, $Q90$, and $Q50$ with positive coordinates. Therefore, for each season, F2 discriminated years with "flat" hydrographs, characterized by stable flows, from years with variable flows and peaks above the infrequent discharge threshold. An illustration of characteristic discharge patterns for each part of the factorial plane is given in Fig. 2*b*.

Which factors can explain changes in trout densities between years?

In all the covariance models we tested, the interaction term was never significant (P values between 0.21 and 0.70), indicating that all significant relationships were statistically shared by all reaches. For any given year, we found no relationship between biological variables, suggesting no intercohort interactions (P values between 0.03 and 0.94). The significant models relating the 0+, 1+, and adult age classes to annual biological variables at year $n - 1$ and hydrological variables are presented in Table 4.

The 0+ fish largely depended on the reach ($R^2 = 63\%$, $P < 0.0001$). The remaining within-reach variability only accounted for 37% of the total 0+ variability. Amongst the hydrological variables, only one significant relationship was found with the first PCA axis during the emergence period ($F1_{emergence}$, $P = 0.0002$), which explained 11.2% of the within-reach variability. The negative slope indicated that when $F1_{emergence}$ increased within reach, i.e., the water level increased, the abundance of 0+ fish decreased. The model was slightly improved when we used a representative variable of $F1_{emergence}$ instead of $F1_{emergence}$ itself. Using $Q10_{emergence}$ (because it was highly covariant with F1 and had a zero coordinate on F2), the global model explained up to 69% of the total 0+ variability, with 16.6% of the 0+ within-reach variability ($P < 0.0001$) caused by the hydrological variable. Because our study sites were roughly grouped in three regions that could possibly experience different hydrological patterns, we checked that the clustering in three regions did not change our findings: there was no difference in 0+ density among regions, and the effect of $Q10_{emergence}$ during the emergence period was similar across the three regions ($P_{region} = 0.210$, $P_{interaction} = 0.288$). The 0+ fish did not exhibit any relationships with either the 0+ fish one year before or the adult fish one year before. However, the 0+ fish were negatively influenced by the abundance of 1+ fish the previous year (i.e., 11.7% of the within-reach variability, $P = 0.0001$).

The variability in the 1+ fish abundances was largely due to differences between reaches ($R^2 = 67\%$, $P < 0.0001$). No relationships were found between the 1+ fish and the hydrological variables. Concerning the biological variables, only the 0+ fish abundances the previous year had a significant positive effect on the 1+ fish ($P < 0.0001$). This meant that, within a reach, the 0+ fish abundance in year $n - 1$ accounted for 20.7% of the variability in 1+ fish abundance in year n.

Abundances of adult brown trout strongly depended on the reach ($R^2 = 72\%$, $P < 0.0001$); therefore, the interannual within-reach variability was low (28%). Adult fish were not related to any hydrological variables, but were highly related to the abundance of 1+ fish the previous year ($P < 0.0001$). Within a reach, the abundance of 1+ fish explained 22.4% of the variability in adult fish abundances.

Was the 0+ fish survival density dependent?

Without simulating the sampling bias, the model relating the 1+ fish density to the 0+ fish density the previous year had a slope b significantly smaller than 1 ($b = 0.330$, $P < 0.0001$; Table 4), therefore suggesting density dependence within the 0+ cohort survival. To test for the potential influence of measurement errors, we successively increased the 0+ densities of 5, 10, 15, and 20 fish·m^{-2}, and the 1+ density of 0, 5, 10, and 15 fish·m^{-2}. Because the sampling error is size dependent (Bohlin et al. 1989), we increased the 0+ density more than the 1+ density in each simulation. With simulated bias, all models had a slope parameter b with a 95% confidence upper limit significantly lower than 1, except for the model simulating the more extreme bias (i.e., no bias on the 1+ fish, increase of 20 0+ fish·100 m^{-2}). Whatever the simulated bias, the effect of the 0+ fish density the

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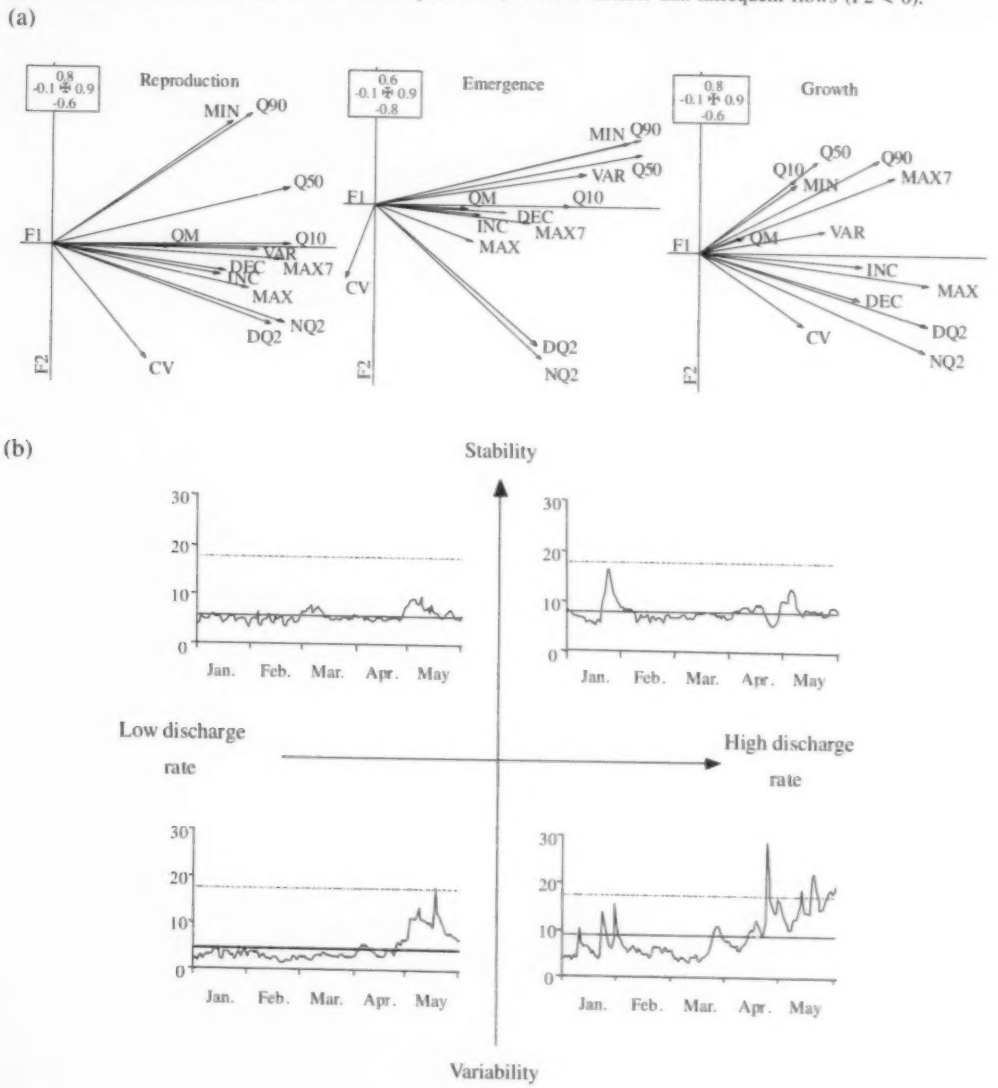
Fig. 2. (*a*) Within-reach PCA (factorial plane F1 × F2) of hydrological variables performed for the spawning, emergence, and growth periods. Definitions of variables are given in Table 3. For each season, insets represent the scale on the F1 and F2 axis. (*b*) Synthetic representation of typical hydrographs after the within-reach PCA analysis. This example is given for a single-gauged station during the emergence period that exhibited different hydrological characteristics across years. On the hydrographs, the horizontal solid line represents the mean discharge over the season for the considered year, the horizontal broken line defining the "infrequent" high flow threshold for this gauged station. The two hydrographs with negative F1 coordinates are characterized by low mean discharges, but the shape of the signal can be "flat" (F2 > 0) or "variable" (F2 < 0). Conversely, the hydrographs with positive F1 coordinates exhibited a high mean discharge over the season, either with little variability (F2 > 0) or with variable and infrequent flows (F2 < 0).



previous year remained highly significant ($P < 0.0001$), and the relationship was statistically similar between reaches.

Was the 0+ fish survival affected by the hydrological conditions?

The models relating the 1+ fish density to the 0+ fish density the previous year were not significantly improved when a hydrological covariate was added (probability for the covariate was always $P > 0.03$).

Discussion

This study aimed to reveal general relationships (i.e., statistically shared by various reaches) between brown trout

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Table 4. ANCOVA models relating the 0+, 1+, and adult densities at year n to the hydrological variables (PCA axes by season) and the 0+, 1+, and adult densities at year $n - 1$.

Dependent	Covariate	Slope b (P value)	Variance covariate (within reach) (%)	Total variance model (%)
0+	None	–(<0.0001)	—	63.3
	$F1_{emergence}$	–0.105 (0.0002)	11.2	67.4
	$Q10_{emergence}$*	–0.732 (<0.0001)	16.6	69.4
	$1+_{n-1}$	–0.503 (0.0001)	11.7	67.6
1+	None	–(<0.0001)	—	66.6
	$0+_{n-1}$	0.330 (<0.0001)	20.7	73.5
Adults	None	–(<0.0001)	—	72.3
	$1+_{n-1}$	0.366 (<0.0001)	22.4	78.5

Note: For each age class, a model without covariate (i.e., equal to an ANOVA with the reach as categorical variable) is first tested to estimate the between-reach variability. The significant threshold for the influence of the covariate is Bonferroni adjusted; therefore, probabilities were considered significant below 0.0017. Listed for each model are the slope for the covariate effect b and its associated corrected probability, the within-reach variability accounted for by the covariate, and total variance explained by the model. Only significant models are presented.

*Model built with a representative variable of the PCA axis (i.e., $Q10_{emergence}$).

age class densities at year n, year-to-year seasonal hydrology, and brown trout age class densities at year $n - 1$. We found only one reliable relationship between trout variables and the seasonal hydrology. The 0+ fish density was strongly and negatively related to the discharge rate during the emergence period, i.e., the higher the discharge during this period, the lower the 0+ fish density. This relationship was statistically comparable across the 30 tested reaches, and was slightly improved when the discharge value exceeded 10% of the time during the emergence period was considered a representative characteristic of the discharge rate. This confirmed previous results obtained on fewer sites. Using mean monthly discharges, Nehring and Anderson (1993) reported similar effects in a study of 11 Colorado streams, as did Nuhfer et al. (1994) in a Michigan stream. Clark (1992) concluded that the recruitment of Arctic grayling was negatively affected by high discharges during the critical spawning, emergence, and larval periods. However, the exact mechanism that leads high discharges to reduce 0+ fish density is not well identified. A possible cause is the flushing of the 0+ fish as a consequence of their inability to maintain their stream position or to find shelters when the water velocities increase during high discharges. Heggenes and Traaen (1988) showed that brown trout larvae entering the free-feeding stage are sensitive to water velocities for a few weeks, and are not able to resist velocities above 25 $cm \cdot s^{-1}$. Although their sensitivity declines as they grow, they are only able to withstand velocities up to 50 $cm \cdot s^{-1}$ on average after two months. Less extreme water velocities, although not displacing fish downstream, may drive young trout to starve in nonoptimal habitats, with higher energy costs or lower food availability, therefore possibly leading to mortality (Elwood and Waters 1969). Other studies have related the mechanistic destruction of eggs or newly hatched fish by substrate movements under high discharges (DeVries 1997 and references therein). Nonetheless, given the average egg clutch burial depth for brown trout (typically between 10 cm and 15 cm, DeVries 1997), we think that only the more extreme discharges could lead to a substantial destruction of eggs. Lapointe et al. (2000) estimated the probability of salmonid egg clutch scour owing to frequent flood (recurrence period <10 years) to be 5%. This probability reached up to 20% for an event recurring less frequently than once every 100 years. Other high-discharge-related geomorphic processes, such as fine sediment fill over the redds and subsequent decrease in oxygen content, can also be detrimental for egg survival (Sear 1993).

Our analysis faced the difficulty of precisely determining which discharge characteristic best explained variation in 0+ densities, because most hydrological descriptors covaried together. In this context, PCA analysis was useful for synthesizing the hydrological pattern into a few independent variables. We found that the within-reaches hydrological pattern could be summarized by a discharge rate axis (F1) and a variability axis (F2), the latter representing the shape of the hydrological signal. Our results indicated that, within reaches, it was the discharge rate during the emergence period, but not the shape of the hydrological signal, that influenced the 0+ cohort size. In other words, for a given discharge level, a hydrograph or flow regime that was either stable or highly variable had the same influence on trout densities (in the range of hydrological variability encountered in our study reaches).

To better quantify this effect, the model relating the 0+ fish density to the discharge value exceeded 10% of the time during the emergence period predicted that, on average between reaches, there would be a loss of about 4 fish·100 m^{-2} (i.e., on average, 28% of the 0+ fish) for a rise in $Q10$ from 1 to 2 times the interannual median discharge (Fig. 3). This loss would be equal to 8 fish·100 m^{-2} (52%) for a rise from 1 to 4 times the interannual median discharge. Although largely significant, the seasonal hydrology only explained a small proportion of variability in 0+ fish density (16.6% of the within-reaches variability). However, fish sampling biases and subsequent errors in the calculation of densities induced noise in the dependent variable, which is known to reduce the slope parameter estimation in statistical models (Snedecor and Cochran 1967). Uncertainties in the calculation of hydrological variables caused by the distance between the fish reach and the gauging station could also have

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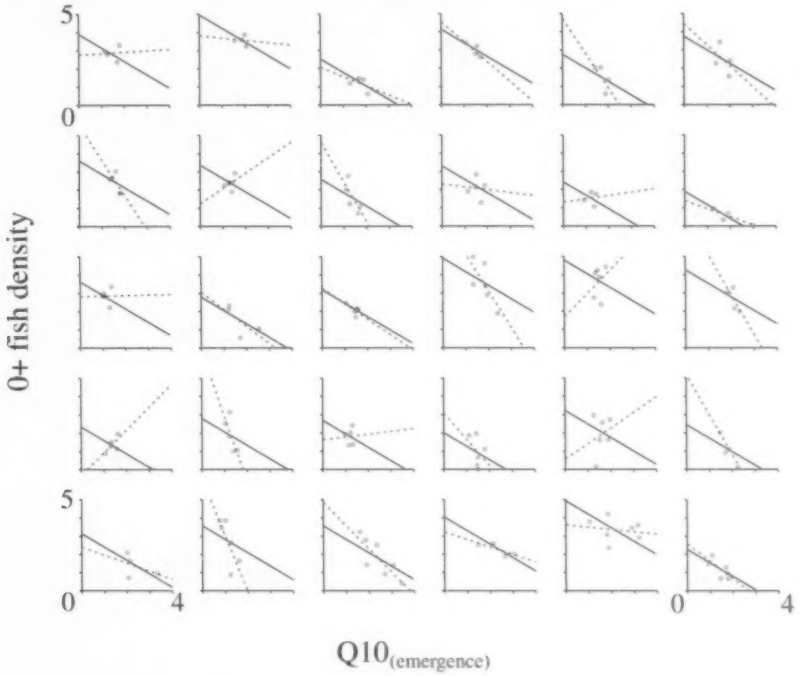
Fig. 3. Relationships between the 0+ fish density and the hydrological variable $Q10$ during the emergence period, detailed for the 30 reaches. Broken lines represent the regression line for the reach; solid lines represented the fit according to the analysis of covariance. The slope is common to all reaches and the intercept depends on the reach.



an effect. It therefore seems likely that the influence of the seasonal hydrology on the 0+ fish density within reaches is stronger than that reported herein.

The influence of the year-to-year seasonal hydrology has also been investigated in nonsalmonid fish. The early 0+ stage represents a critical period of fish development, where abiotic (e.g., hydrological) conditions are of primary importance. In small streams, spring floods were reported to greatly reduce the 0+ cyprinid and centrarchid abundances (Schlosser and Angermeier 1990). In a larger stream, Cattanéo et al. (2001) found that the annual changes in abundance of 10 cyprinid species were related to the hydrological conditions during the spawning period, in agreement with the biological traits of these species. Such studies were, however, descriptive, and did not really quantify the influence of hydrology.

The other significant models only involved biological variables. The 1+ density was strongly related to the 0+ density the previous year, similarly across reaches, as was the adult density with the 1+ density the previous year. These relationships, widely found in population dynamics, reflected the follow-up of the cohorts from one year to the next. As for the 0+ densities, both the 1+ and adult densities were highly dependent on the reach (R^2 = 67% and 72%, respectively). Within reaches, the knowledge of the 0+ or 1+ densities at year n explained about 20% of the variability of these cohorts at year $n + 1$. This proportion is rather small. Nelson (1986) estimated that the size of a year class explained between 63% and 93% of the variation in density of the cohort the next year. However, these estimates were based on older trout (>2+), whose year class strength varies less between years than younger fish (Jowett 1995).

Our results also suggested density dependence in the 0+ survival function, unless we assumed a sampling bias of 20 0+ fish·100 m^{-2} and no bias on the 1+ densities. However, this particular model was unlikely to reflect a reliable sampling bias for two main reasons: first, there was certainly an error in estimating the 1+ fish density, and second, this bias on the 0+ densities would mean that we only caught about 40% of the 0+ fish (their average density in samples was 13.8 fish·100 m^{-2}), which was improbable given that they were readily catchable at the time of sampling. Density dependence in the young-of-the-year survival is a somewhat common feature of salmonid populations. Intracohort density-dependent mortality has been shown to occur during the first few months of life (Le Cren 1973; Mortensen 1977), and has been largely attributed to competition for limited resources, such as food and space (Le Cren 1973). Mortensen (1977) suggested that a physically complex habitat, which would prevent 0+ fish from seeing one another, would reduce mortality owing to territorial competition. Competition for food is expected to play a role in the amount of energy stored by the 0+ fish before the overwintering period, during which they survive primarily on their energy reserves (Cunjak 1988). As a result, insufficient reserves associated with reduced food intakes could partly explain the 0+ mortality during the winter (Cunjak 1988). The discharge pattern did not influence the survival of the 0+ cohort. This reinforces the

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idea that the 0+ fish are sensitive to the hydrological pattern during a limited critical period, after which they become able to cope with flow fluctuations. Similarly, Hayes (1995) reported that a large flood six months after emergence had no apparent effect on the 0+ trout (80–100 mm in length) in a New Zealand river. Mesick (1995) found a negative correlation between winter stream flows and the 1+ trout survival index, but no relationship with the 0+ survival.

Strikingly, we found a significant negative relationship between the 0+ fish at year *n* and the 1+ fish the previous year, statistically shared by all reaches. To our knowledge, a similar relationship has not been demonstrated elsewhere. We would rather have expected a negative relationship between the 0+ fish at year *n* and the 1+ fish during the same year, as reported by Bohlin (1977). It is difficult to find a sound biological reason to account for this relationship. We can only speculate the following. (*i*) The microhabitat used by the 0+ fish largely overlaps that used by 1+ fish (Baglinière and Maisse 1991), after which a change in microhabitat use appears when the fish becomes 2+. At the time of sampling, the 1+ fish of the previous year just changed their microhabitat use, but there could have been some interactions (competition or predation) with the 0+ fish that searched for key positions. (*ii*) High densities within a reach were mainly due to the 0+ and 1+ fish, so it is possible that high densities of 1+ fish might disturb spawning, and therefore lead to a low 0+ cohort the next year. This relationship should therefore be tested in further studies to confirm whether it is reliable or not. We observed no stock–recruitment relationship between the reproductive pool size (adult density) and the 0+ density the following year, conversely to Elliott (1989). This suggested that even a low density of adults could produce a large 0+ cohort the next year, as already mentioned by Le Cren (1973).

Our results suffered from some limitations, inherent in the use of large data sets. First, although the fishing procedure was standardized, variability in the fishing efficiency could not be avoided. Second, using data collected by a large number of people over many sites prevented any precise knowledge of each sampled reach or sampling campaign, which may lead to a loss of important information useful for the interpretation of the results. Overall, using large data sets results in a coarse grain data quality collected in numerous samples compared to the fine grain quality of smaller sample sizes.

In summary, we demonstrated that both the hydrological and biological forces constrain trout population dynamics, mainly during the first year of life. Owing to their complex life histories, freshwater fish are sensitive to abiotic and biotic factors at different developmental stages. These factors can operate simultaneously, but the predominance of each can be more marked at a particular life stage. We found that the 0+ densities were first regulated by the discharge pattern during the emergence period. After this period, we found that a density-dependent regulation occurred during the first year of life, during which the hydrological pattern did not have any impact on fish survival. Thereafter, classical follow-up of cohorts was shown, but the hydrological pattern did not influence either the 1+ fish or the adults. In terms of trout population management, these results suggested that attention should be paid to limiting the flow during the emergence period in regulated streams, possibly by avoiding large flow releases downstream from reservoirs. Here, we focused on the transferability of relationships between hydrology and biotic interactions and trout dynamics observed within reaches. It is important to note, however, that trout populations mainly exhibited variability in space (between-reaches variability between 63% and 72%) rather than temporal fluctuations between years within reaches. A high level of between-reach variability has been observed elsewhere (Hayes 1995; Jowett 1995) and associated with various environmental and biotic features, such as the reach gradient, the width, the cover, the streambed sediment, the availability of spawning habitat, or the benthic invertebrate biomass (see Fausch et al. 1988; Beard and Carline 1991; Jowett 1995). Our within-reaches study may be useful for identifying the major hydrological variables that could be included in between-reaches studies; the hydrological variables, within a particular season, that systematically affect population dynamics may be good candidates for explaining between-reaches differences in population structure.

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Estimation of short-term centers of activity from an array of omnidirectional hydrophones and its use in studying animal movements

Colin A. Simpfendorfer, Michelle R. Heupel, and Robert E. Hueter

Abstract: A method for studying animal movements based on data from independent data-logging acoustic receivers is described. The method takes presence or absence data from multiple receivers arranged in an array and converts them to position estimates based on weighted means of the number of signal receptions at each receiver during a specified time period. The method is equivalent to a short-term center of activity rather than a precise estimate of location at a single time. The utility of the method was assessed using data from a study of neonate blacktip sharks (*Carcharhinus limbatus*). Periods between 5 and 60 min were tested to find the most appropriate interval for estimating positions. The results from the method agreed closely with a simulated shark track and data from actively tracked sharks. The median distances between successive locations from the mean-position algorithm were between 28% and 42% of those from active tracking because of the center-of-activity nature of the method. The results presented demonstrate that the technique provides a useful method for investigating long-term movement patterns, space utilization patterns over broader areas, and home range.

Résumé : On trouvera ici une méthode pour étudier les déplacements des animaux à l'aide d'enregistrements de données par des récepteurs acoustiques indépendants. Il s'agit de prendre des données de présence ou absence provenant de plusieurs récepteurs installées en réseau et de les convertir en estimations de position d'après les moyennes pondérées des nombres de signaux reçus à chaque récepteur pendant une période de temps donnée. Cela équivaut à déterminer le centre d'activité à court terme plutôt que la localisation exacte à un moment précis. L'utilité de la méthode a été examinée à l'aide des données d'une étude sur des Requins bordés (*Carcharhinus limbatus*) néonates. Des périodes de 5 à 60 min ont été évaluées pour fixer l'intervalle approprié pour l'estimation des positions. Les résultats obtenus s'accordent bien avec un déplacement simulé de requin et avec des données sur des requins suivis de façon active. Les distances médianes entre deux positions successives obtenues par l'algorithme de position médiane correspondent à 28–42 % de celles obtenues par dépistage actif, parce que la méthode est basée sur les centres d'activité. Nos résultats démontrent que la technique est utile pour étudier les patterns de déplacement à long terme, l'utilisation de l'espace sur des surfaces inportantes et le domaine vital.

[Traduit par la Rédaction]

Introduction

Tracking the movements of fish and other aquatic animals has most often been achieved by active tracking, where an individual is fitted with an acoustic transmitter and followed for as long as possible and its position is estimated at regular intervals (Nelson 1990). Longer-term studies using manual telemetry have been undertaken by returning to the study site at regular intervals and relocating the tagged animal (e.g., Morrissey and Gruber 1993; Baras 1998). Recent advances in acoustic receiver technology have made automated submerged data-logging instruments available that permit automated collection of long-term data (e.g., Cote et al. 1998; Heupel and Hueter 2001; Voegeli et al. 2001). All of these units employ omnidirectional hydrophones but have differing abilities to estimate position of animals carrying acoustic transmitters. The most advanced of these units are radio or cable linked and controlled by a base-station computer (Voegeli et al. 2001). These systems provide a position estimate accurate to within a few metres based on the time of signal arrival at each of the receivers. Single-frequency, independent units have also been developed that do not provide position estimates. Instead, these units report the presence of an animal fitted with a transmitter within the range of the receiver along with the date and time of signal reception (Heupel and Hueter 2001; Voegeli et al. 2001). Both styles of receiver allow continuous tracking of animals for much longer periods than traditional active-tracking efforts, provided that they stay within the range of the receivers.

Researchers planning telemetry studies are faced with deciding which style of system to use. The advanced linked receivers provide accurate position data but cost significantly more than independent, single-frequency receivers and are often limited by the number of receivers that can be linked in the array. The location estimates are mostly limited to the area between the three or more receivers (Klimley et al. 2001; Voegeli et al. 2001). If animals swim outside the range

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C.A. Simpfendorfer,[1] M.R. Heupel, and R.E. Hueter.
Center for Shark Research, Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, U.S.A.

[1]Corresponding author (e-mail: colins@mote.org).

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of one of the linked receivers, triangulation is impossible. Alternatively, by using the single-frequency inexpensive receivers, a researcher can provide acoustic coverage of a much larger area. This approach ensures better long-term coverage of mobile animals, with the trade-off being that position data have been limited to presence or absence within the omnidirectional range of each receiver. The solution to the decision about which receiver style to use requires the researcher to solve the accuracy–coverage–cost trade-off for greatest benefit.

The ability to estimate an animal's location within an array more accurately than simple presence or absence at a given receiver would enhance the information gathered from independent style receivers. It would also allow for more detailed analysis of animal movement patterns, which to date have been restricted to changes in broad spatial patterns (e.g., Heupel and Hueter 2001). In this paper, we demonstrate a method for estimating position and movements based on short-term centers of activity calculated from data collected by an array of independent, single-frequency omnidirectional receivers. We illustrate this method using data from a study of neonate blacktip sharks (*Carcharhinus limbatus*) in a summer nursery area and compare the results to those obtained by active-tracking techniques.

Materials and methods

Position estimation algorithm

For a single omnidirectional hydrophone, the probability that a signal from a transmitter will be logged is proportional to the distance between the receiver and the transmitter. In the simplest case, the probability of reception is linearly related to distance and the probability surface would be conical in shape. Thus, over a period of time (Δt), the number of receptions would be greater the closer the signal source is to the receiver. It is this case that will be considered in this paper.

In the case where there are multiple receivers with overlapping detection ranges, and each receiver logs a signal, the shape of the probability surface for the signal's source location can also be approximated by a cone, but centered on a point equidistant from each receiver. Thus, over Δt, the number of receptions at each receiver will be equal if the signal source is located equidistant from each receiver.

In the same array of receivers, if the number of signal receptions is not equal, then it can be assumed that the signal source is closer to the receiver(s) that has the greatest number of receptions. Thus, in an X- and Y-coordinate system (e.g., latitude and longitude), the best estimate of the position of the source of the signal is the mean of the receiver locations weighted by the number of receptions. One implication of this method is that the mean-position estimate will always fall within the minimum convex polygon (MCP) described by the receiver locations. Most accurate results will thus be obtained by designing the receiver array such that animals will rarely, if ever, occur outside the array's MCP.

In the situation of mobile animals, the mean-position estimate will only be constant if there is limited movement during Δt. This can be achieved if Δt is small relative to the speed of the animal or if movement results in limited change in position (e.g., movement pattern that involves regular turning to remain in the same area). Selection of an appropriate Δt is required for accurate results from this method and must balance the need to record sufficient signal receptions from more distant receivers against the need to not allow the position of the animal to change too much.

In many systems using independent, single-frequency omnidirectional receivers, signals are sent infrequently (once every 1–2 min) so that multiple transmitters can be used (Voegeli et al. 2001). In these situations, Δt will probably need to be in the range of 5 to 60 min to allow sufficient signal receptions. This means that there would be a significant level of movement for mobile animals. As such, the mean-position estimate will not be equivalent to a point estimate (such as those made by receiver arrays that use signal-arrival timing methods); instead, the mean-position estimate is equivalent to a short-term center of activity for the period Δt (see Worton (1987) for a review of center of activity as part of a review of home-range models).

Practical example—neonate blacktip sharks in a summer nursery area

To test the use of the mean-position algorithm as a method of estimating animal centers of activity and movements, we applied it to a study of neonate blacktip sharks (*Carcharhinus limbatus*) in a summer nursery area in Terra Ceia Bay, Florida. Neonate *C. limbatus* occur in this bay from May to November before migrating southwards during the winter. A study of the movements of these animals within Terra Ceia Bay was instituted in 1999 (Heupel and Hueter 2001) using an array of 14 Vemco VR1 (Vemco Ltd., Shad Bay, N.S.) omnidirectional hydrophones. In 2000, the study was expanded to include an additional 10 hydrophones to increase the acoustic coverage in areas surrounding the bay (Fig. 1). Methods of deploying and maintaining the hydrophones are described in Heupel and Hueter (2001).

Neonate *C. limbatus* were caught using rod and reel and were fitted internally with Vemco V16 RCODE acoustic transmitters. The RCODE transmitters were programmed to send a coded signal at a random interval between 45 and 75 s. Details of the surgery to fit transmitters is given in Heupel and Hueter (2001). In 1999, 18 *C. limbatus* were released with transmitters, with animals residing in Terra Ceia Bay for periods between 3 and 159 days. In 2000, 33 specimens were released and stayed in the bay between 1 and 167 days. In addition to the animals monitored by the array of VR1 receivers, seven neonate *C. limbatus* were tracked during 1999 and 2000 using standard manual techniques with positions estimated every 15 min. Positions were estimated by taking bearings from the vessel to the animal and recording the receiver gain at which the signal could no longer be detected. Distance from the vessel to the signal source was then estimated based on range tests of transmitters.

Testing the assumption

To test the assumption that the number of receptions decreases within increasing distance from a receiver, a static test was performed in the study site. Transmitters were moored at eight known distances from a receiver for 4 days, and the number of receptions was determined per day for each distance. Daily numbers of receptions were used, as the random signal interval of the transmitters requires a sufficient period to assume that equal numbers of signals have been sent. A

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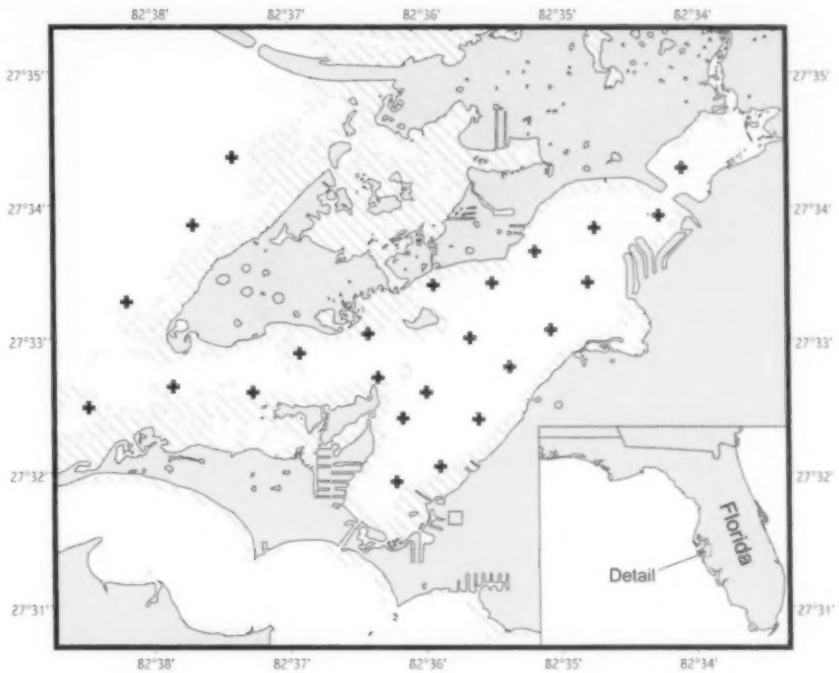
Fig. 1. Location of automated data-logging acoustic receivers (+) in Terra Ceia Bay, Florida, U.S.A. Cross-hatched areas indicate seagrass habitats.



linear regression was fitted to the numbers of daily receptions and distance to test for a significant relationship.

Data analysis

To explore the usefulness of the mean-position algorithm in studying the movement patterns of animals, the methodology was explored using the data set from the Terra Ceia Bay *C. limbatus* study and compared with results from actively tracked animals. A number of different aspects of the mean-position algorithm were explored to investigate its usefulness. Does the method of calculating the mean influence the results? What is an appropriate value of Δt? And, how do the estimates of distance and bearing between successive locations compare with those from active tracking?

Calculation of mean position

Because *C. limbatus* are constantly swimming, the positions estimated by the algorithm represent a center-of-activity type of location. Two methods of estimating the mean were evaluated: arithmetic and harmonic (Table 1). Differences in the positions between these techniques were calculated and plotted as frequency distributions.

Size of Δt

To evaluate the appropriate level of Δt for the blacktip shark data, two analyses were undertaken. Firstly, the number of different stations at which a signal was received for a range of Δt values (5, 10, 15, 20, 30, 60 min) was determined. More accurate estimates of position are made when greater numbers of stations receive signals during Δt. Secondly, the total number of signal receptions across all receivers during Δt was determined. Again, position accuracy is greater the larger the number of signal receptions during Δt. These analyses were undertaken for two animals that resided in the Terra Ceia Bay study site for longer than 100 days.

Table 1. Formulae for calculating weighted means ($\bar{X}_{\Delta t}$, $\bar{Y}_{\Delta t}$) using arithmetic and harmonic approaches within an *X*- and *Y*-coordinate system.

Mean type	*X* coordinate	*Y* coordinate
Arithmetic	$\bar{X}_{\Delta t} = \frac{\sum_{i=1}^{n} R_i X_i}{\sum_{i=1}^{n} R_i}$	$\bar{Y}_{\Delta t} = \frac{\sum_{i=1}^{n} R_i Y_i}{\sum_{i=1}^{n} R_i}$
Harmonic	$\bar{X}_{\Delta t} = \frac{\sum_{i=1}^{n} R_i}{\left(\sum_{i=1}^{n}\left(1 \Big/ \sum_{i=1}^{n} R_i X_i\right)\right)}$	$\bar{Y}_{\Delta t} = \frac{\sum_{i=1}^{n} R_i}{\left(\sum_{i=1}^{n}\left(1 \Big/ \sum_{i=1}^{n} R_i Y_i\right)\right)}$

Note: n, the number of receivers in the array; R_i, the number of receptions at the ith receiver during Δt; X_i, the X coordinate of the ith receiver; Y_i, the Y coordinate of the ith receiver.

Distance between positions

The distance and bearing between successive position estimates were calculated using great circle formulae. Distances and bearings were calculated for six sharks tracked for over 100 days during the summer of 2000. Distances were calculated for Δt values of 15, 30, and 60 min. Distance and bearing values were compared with those from

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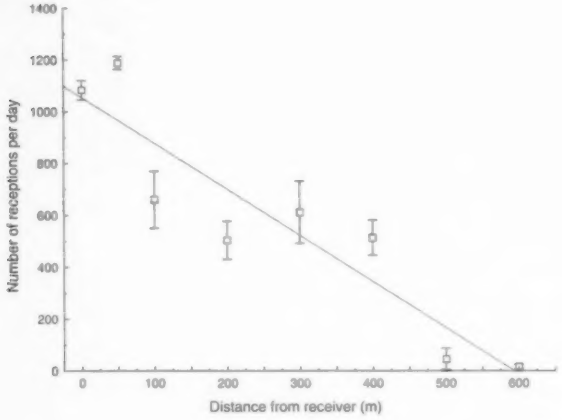
Fig. 2. Relationship between the number of signals logged by a receiver and the distance from the receiver. Squares represent mean daily values for 4 days and error bars represent ±1 standard error.



active tracks of four animals tracked during the summer of 2000. Values of distance and bearing from active tracks for 30- and 60-min intervals were calculated by taking every second and fourth value, respectively. Distances were calculated in metres and compared between methods and Δt values using the nonparametric Kruskal–Wallis analysis of variance (ANOVA). Distributions of bearings were tested for uniformity using Rayleigh's test. Where distributions of bearings were not uniform, they were compared between methods and Δt values using paired χ^2 tests.

Comparison to simulated shark track

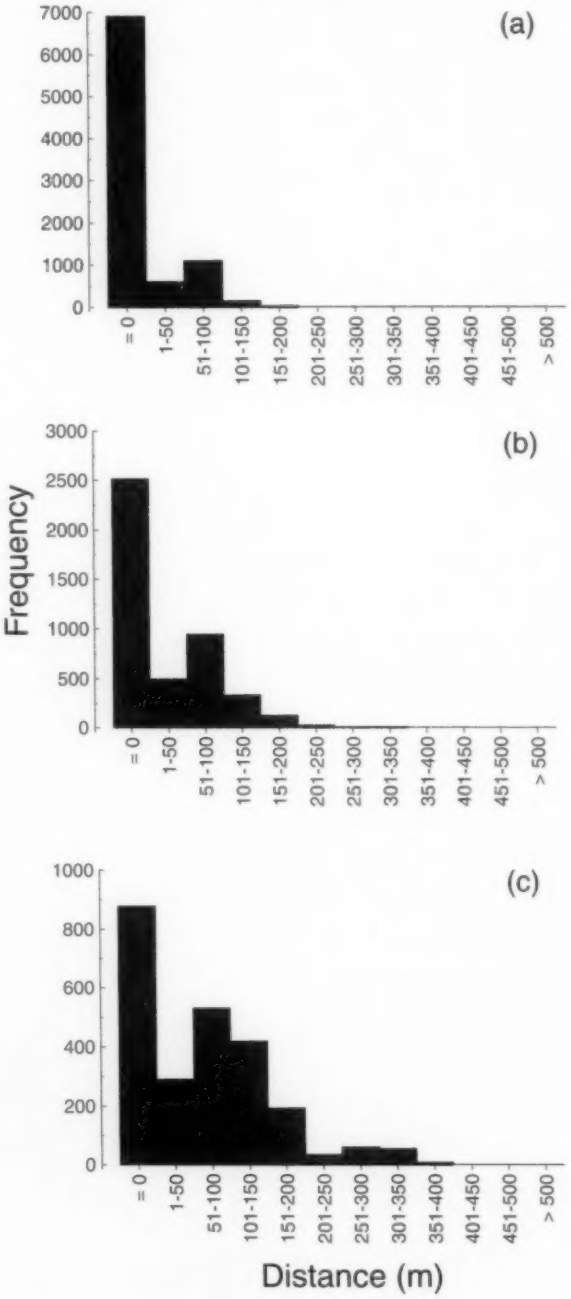
Because of the small size of *C. limbatus* used in this study, it was not possible to attach a tag for both active tracking and passive monitoring. Thus, to assess the accuracy of the mean-position algorithm, estimated locations were calculated for a simulated shark track. The track was created by towing a submerged transmitter behind a small vessel powered by an electric trolling motor. The use of the trolling motor eliminated acoustic interference from an outboard motor. The position of the vessel was recorded every 15 min using a Global Positioning System (GPS). The simulated track lasted approximately 6 h (the limit of the trolling-motor batteries). Mean-position estimates were calculated for Δt = 15 and 30 min. The vessel track and estimated positions were plotted, and the distance between mean-position estimates and the track was calculated using the great circle formula.

Results

Testing the assumption

There was a significant negative linear relationship between the daily number of receptions and the distance from a receiver (Fig. 2; $R^2 = 0.78$, $p < 0.0001$). The assumption of linearity that underlies this methodology was thus supported for this equipment within Terra Ceia Bay.

Fig. 3. Distances between arithmetic and harmonic mean estimates of location for three values of Δt: (*a*) 15 min, (*b*) 30 min, and (*c*) 60 min. Data based on two blacktip sharks resident for over 100 days in Terra Ceia Bay during 2000.



Comparison of mean calculations

The use of arithmetic and harmonic mean in the mean-position algorithm produced very similar results. In most

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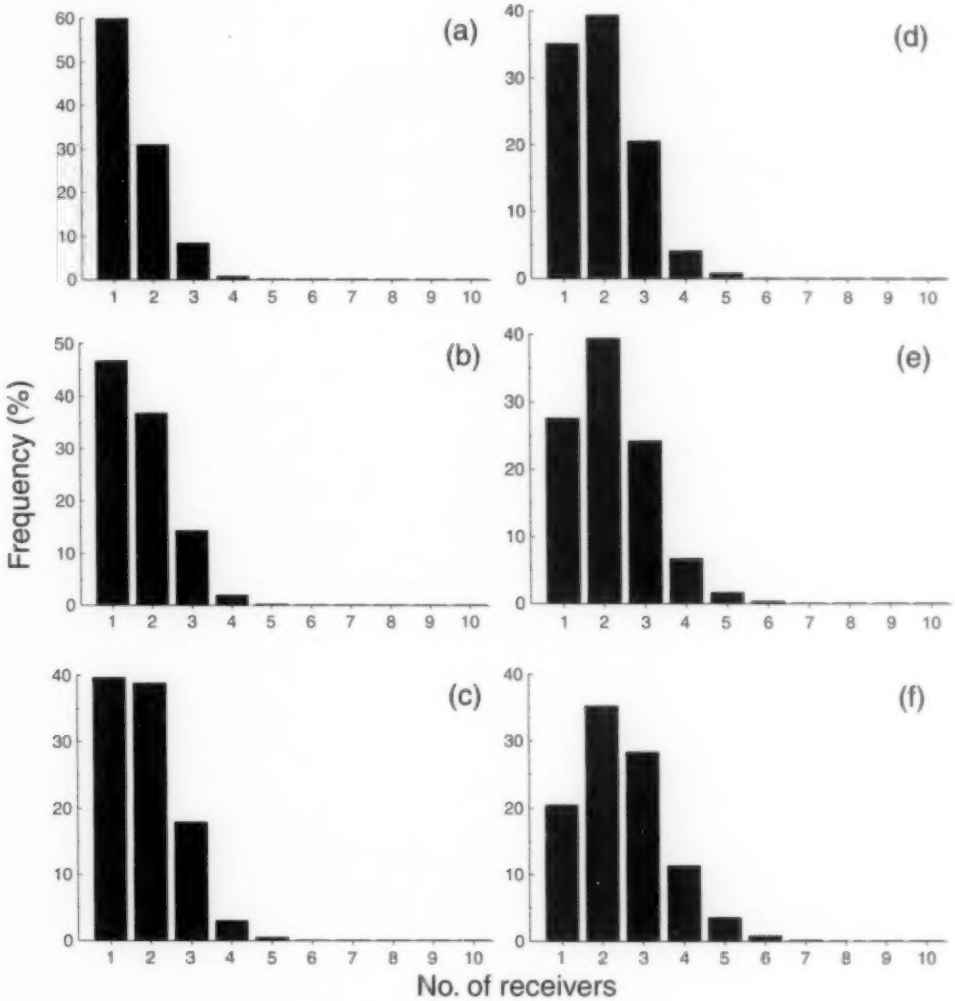
Fig. 4. Frequency distributions of the number of omnidirectional acoustic receivers logging signals for six values of Δt: (*a*) 5 min, (*b*) 10 min, (*c*) 15 min, (*d*) 20 min, (*e*) 30 min, and (*f*) 60 min. Data based on two blacktip sharks resident for over 100 days in Terra Ceia Bay during 2000.



cases, there was no difference in the position estimated by the two calculation methods (Fig. 3). At all values of Δt, the most frequent differences (when they existed) were between 50 and 100 m. At Δt = 15 and 30 min, most differences were less than 100 m, and none was greater than 200 m (Figs. 3*a*, 3*b*). For Δt = 60 min, most differences were less than 200 m (Fig. 3*c*). Maximum differences increased with increasing Δt but never exceeded 500 m.

Size of Δt

The number of stations receiving signals from an individual tag during each Δt increased with increasing size of Δt (Fig. 4). The variability in the number of stations receiving a signal also increased with increasing Δt. When Δt = 5 min, about 60% of periods had only one station receiving a signal; at Δt = 15 min, 40% of periods had reception at one station; and at Δt = 60 min, only 20% of periods had reception at one station. At $\Delta t \geq 20$ min, the most frequent number of receptions during periods was greater than 1.

The number of signal receptions (across all receivers) per Δt period changed considerably with increasing Δt (Fig. 5). At Δt = 5 min, there were always less than 10 signal receptions, with less than 5 receptions in most periods. Increasing Δt increased the maximum number of receptions per period. At Δt = 60 min, the distribution was more uniform than for other values. One and two signal receptions were the most common for all values of Δt.

Comparison of mean-position estimates to a simulated shark track

When comparing the simulated shark track and the mean-position estimates based on the data from the track, most points fell close to the simulated track (Fig. 6). The median distance between mean-position estimates and locations taken

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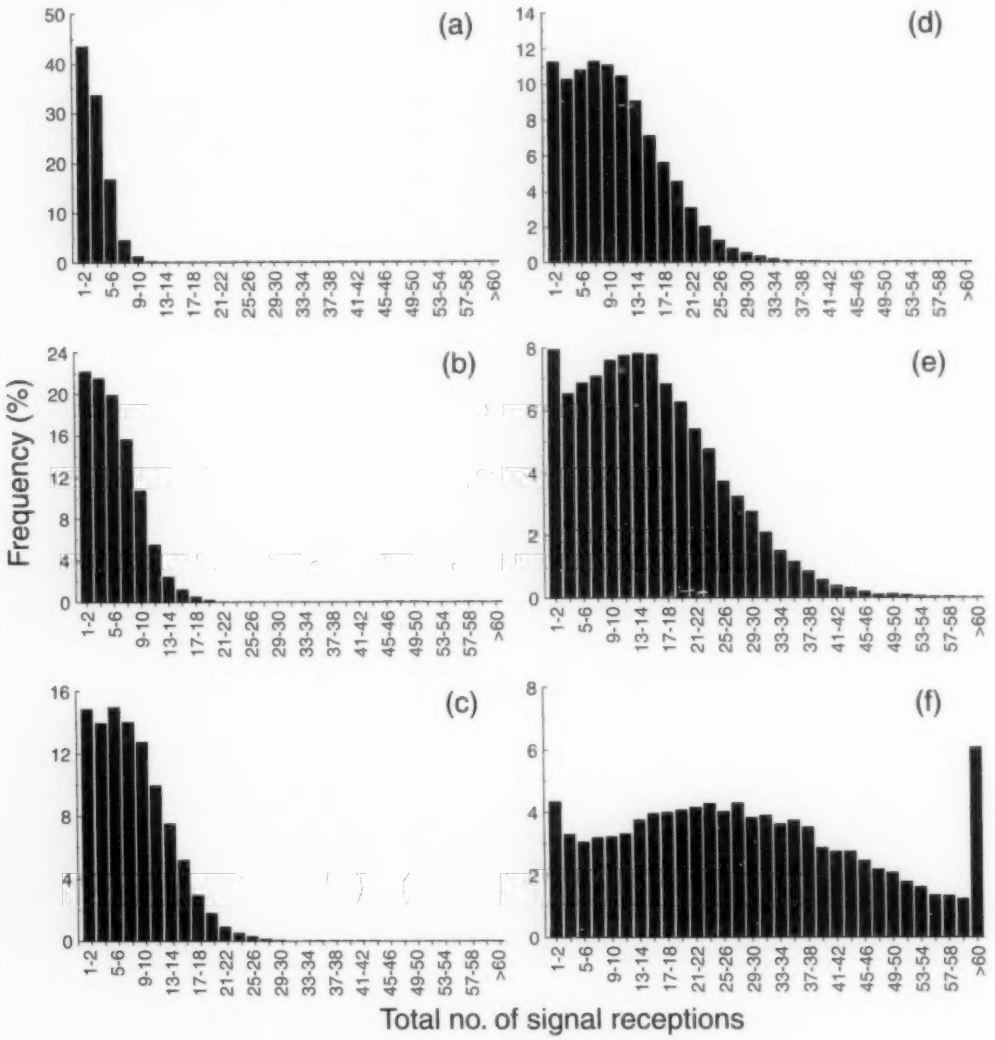
Fig. 5. Frequency distributions of the total number of signal receptions at omnidirectional acoustic receivers for six values of Δt: (*a*) 5 min, (*b*) 10 min, (*c*) 15 min, (*d*) 20 min, (*e*) 30 min, and (*f*) 60 min. Data based on two blacktip sharks resident for over 100 days in Terra Ceia Bay during 2000.



every 15 min by GPS was 209 m for Δt = 15 min and 223 m for Δt = 30 min. Greatest distances between the simulated track and mean-position estimates were observed in the southern region of Terra Ceia Bay, whereas those in the central and northern portions of the bay were relatively small.

Comparison of mean-position estimates to active track data

Position estimates from consecutive Δt periods can be formed into tracks illustrating the movement of an animal, for example, the tracks for two blacktip sharks over a 24-h period on 9 August 2000 (Fig. 7*a*). These two tracks show that sharks utilized confined areas within Terra Ceia Bay. Animal A spent most of its time in an area close to Bird Key, with excursions from its core area along the longitudinal axis of the bay. Animal B maintained a core area northeast of that of animal A but showed few excursions from this area. Both tracks show position estimates that fall along lines joining the locations of two receivers, a result of receptions at only two stations during a Δt period. The tracks were also restricted to the area within the MCP described by the receivers. For comparison, the type of track for a blacktip shark inside Terra Ceia Bay obtained by active tracking is illustrated in Fig. 7*b*. This track is for the same 24-h period shown in Fig. 7*a*. The shark tracked showed a diel difference in the area utilized, maintaining a core area in the northeast of the bay during the day and roaming over a larger area at night to the southwest of the daytime core area. The track shows a utilization pattern that at times ex-

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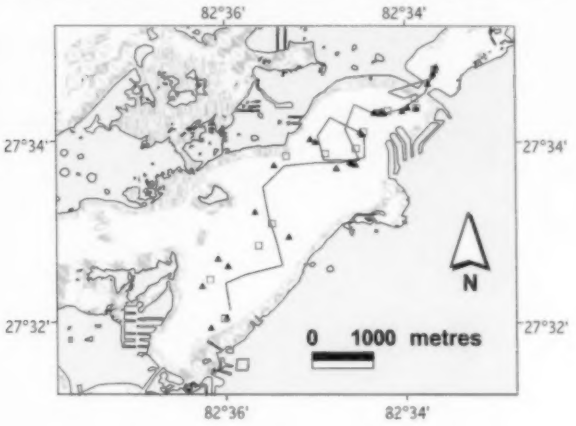
Fig. 6. Mean-position estimates based on Δt = 15 min (solid triangles) and Δt = 30 min (open squares) from a simulated shark track (solid line). Simulated track data were produced by towing a transmitter behind a small vessel powered by an electric trolling motor. Cross-hatched areas indicate seagrass habitat.

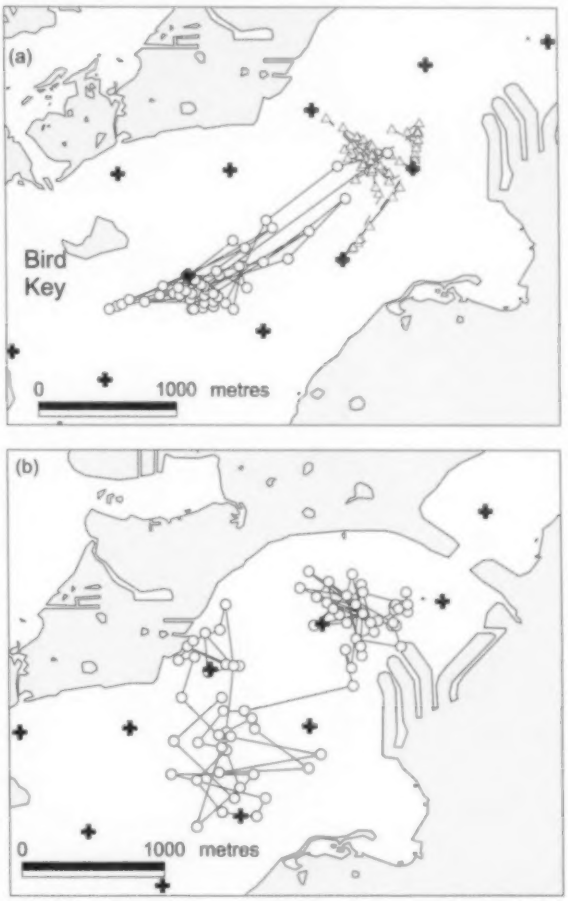


tended to near the edge of the bay, but for most of the tracking period, the estimated locations fell within the MCP described by the receivers.

The distance between successive locations based on the mean-position algorithm was not significantly different between Δt values (Kruskal–Wallis ANOVA, χ^2 = 3.079, p = 0.214; Figs. 8*a*–8*c*). The most noticeable differences were in the proportion of zero values that decreased with increasing Δt. Almost all distances between locations were less than 1000 m, with most between 1 and 200 m. The distance between locations based on active-tracking data was significantly different between different values of Δt (Kruskal–Wallis ANOVA, χ^2 = 40.480, p < 0.001; Figs. 8*d*–8*f*). Distances of 200–400 m between points were the most common for all Δt values. Increasing values of Δt resulted in a greater number of distances greater than 400 m. There were significant differences between the distances from the mean-position algorithm and active tracking at each value of Δt (Kruskal–Wallis ANOVAs: 15 min, χ^2 = 146.84, p < 0.001; 30 min, χ^2 = 209.26, p < 0.001; 60 min, χ^2 = 131.75, p < 0.001). Median values of distance between successive locations based on the mean-position algorithm were between 28 and 42% of those from manually tracked sharks.

Bearings between successive points based on the mean-position algorithm were clumped into several dominant groups (Figs. 9*a*–9*c*), the most frequent of which matched the orientation of the Bay and the longitudinal axis of the receiver array. The distributions of bearings were nonuniform at Δt < 60 min (Rayleigh's test: 15 min, p < 0.001; 30 min, p < 0.001). However, at Δt= 60 min, the distribution of bearings was uniform (Rayleigh's test p = 0.55). Pairwise χ^2 tests showed no differences in the distributions of bearings between Δt values for mean-position data (15 vs. 30 min, χ^2 = 14.47, p = 0.63; 15 vs. 60 min, χ^2 = 16.41, p = 0.49; 30 vs. 60 min, χ^2 = 6.82, p = 0.99). Increasing Δt reduced the dominance of the bearings matching the orientation of the Bay. Bearings based on manually tracked sharks were uniformly distributed (Rayleigh's tests: 15 min, p = 0.75; 30 min, p = 0.81; 60 min, p = 0.55), especially at Δt values of 30 and 60 min (Figs. 9*d*–9*f*). The distributions of bearings were significantly different between mean-position and active-tracking methods at all levels of Δt (pairwise χ^2 tests: 15 min, χ^2 = 189.74, p < 0.001; 30 min, χ^2 = 80.44, p < 0.001; 60 min, χ^2 = 54.15, p < 0.001).

Fig. 7. Examples of movement patterns of blacktip sharks over a 24-h period determined using (*a*) the mean-position algorithm for two individuals within the study site on 9 August 2000 and (*b*) traditional active tracking with position estimated every 15 min for a single individual tracked on 9 August 2000. Mean-position data are based on Δt = 15 min.



Discussion

The advent of relatively inexpensive submersible data-logging acoustic receivers has provided researchers with a useful tool for long-term monitoring of movements of aquatic animals. However, these affordable units are only capable of recording presence or absence data, which to date has limited their usefulness. The results presented in this paper indicate that with the correct arrangement of the acoustic receivers it is possible to provide estimates of the

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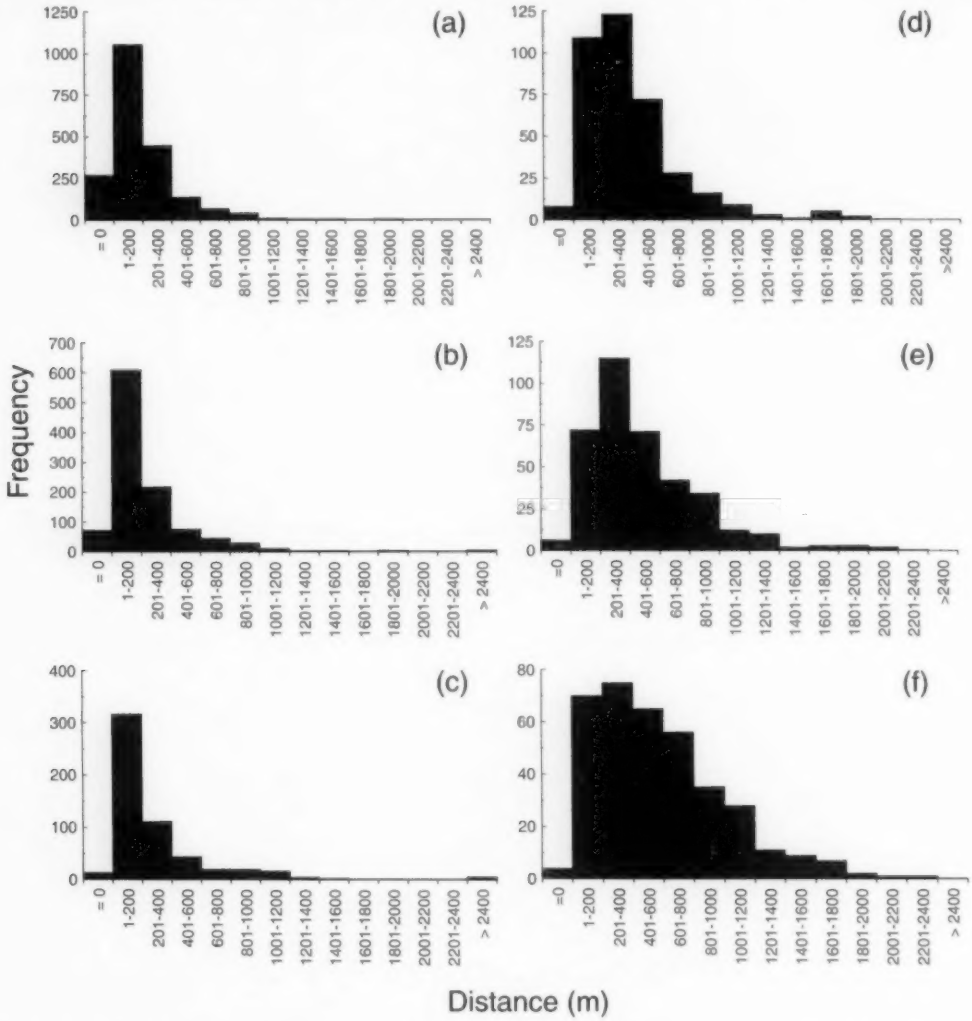
Fig. 8. Frequency distributions of distances between successive location estimates based on the mean-position algorithm (*a–c*) and active tracking (*d–f*). Distributions for three values of Δt are shown: (*a*, *d*) 15 min, (*b*, *e*) 30 min, and (*c*, *f*) 60 min. Data are based on four blacktip sharks resident in Terra Ceia Bay for over 100 days during 2000.



location of an animal at a significantly finer scale than simply the presence within the signal reception range. This type of approach has not been used before in acoustic telemetry studies and provides researchers with an alternative method of studying long-term movements. The advantage of this approach is that the receivers are relatively inexpensive, allowing large arrays to be deployed and significantly increasing the spatial coverage over which tagged animals can be monitored. To date, most studies of long-term movements using acoustic monitoring equipment have used expensive signal reception time systems that have limited spatial ranges. For example, Cote et al. (1998) used an array of four hydrophones to study juvenile cod movements within an area approximately 300 m × 300 m, and Bégout and Lagardère (1995) used a similar system that could operate in a 500-m^2 area to study seabream (*Sparaus aurata* Linneaus). Klimley et al. (2001) describe a system that has a series of three radio-linked hydrophones and had a spatial coverage of approximately 1 km^2. In contrast to these spatial coverages, the mean-position approach can be applied to an array of acoustic receivers of any size, restricting the spatial coverage only to that which the researcher can afford. In the current study, the spatial coverage provided by 24 receivers was approximately 8 km^2.

Many factors can affect the possibility that an acoustic signal will be logged by a receiver, including bottom topography, submerged aquatic vegetation, different sensitivities and powers between pieces of equipment, signal overlap because of large numbers of animals present in an area or short signal-repeat intervals, and noise from biological (e.g., oysters) and human sources (e.g., outboard motors). All of these factors tend to reduce the linearity in the relationship between the number of signals received and the distance from a receiver. Despite the presence of all of these factors within

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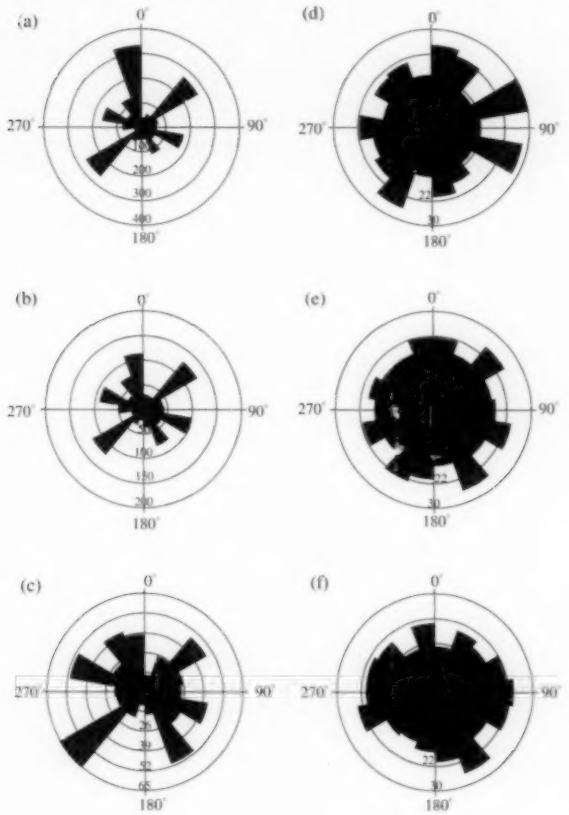
Fig. 9. Frequency distributions of bearings between successive locations from (*a–c*) the mean-position algorithm and (*d–f*) active tracking. Distributions for three values Δt are shown: (*a*, *d*) 15 min, (*b*, *e*) 30 min, and (*c*, *f*) 60 min. Data are based on four blacktip sharks resident in Terra Ceia Bay for over 100 days during 2000.



Terra Ceia Bay, the assumption of linearity was met, supporting the use of the mean-position algorithm described in this paper.

The results of the mean-position algorithm depend heavily on the value of Δt selected. For the blacktip shark, Δt values less than 30 min produced few receptions at multiple receivers, resulting in position estimates based on few data. However, at Δt values of 30 min or greater, position estimates were often based on multiple receptions at multiple receivers. Values of Δt greater than 60 min were not tested in this study because it was believed that the movement of the blacktip sharks would be too great to provide meaningful position estimates and therefore would reduce the ability to study within-day effects in subsequent studies (e.g., changes in behavior resulting from tide or day–night cycles). However, in other studies, Δt values greater than 60 min may be useful or necessary. The use of arithmetic and harmonic mean calculations did not change results to any major extent.

The application of this technique to other studies will require investigators to determine an appropriate value of Δt. This is a result of the best value being dependent upon the frequency of signal transmissions, the rate of animal movement, and the amount of interference at the receivers (either from physical conditions or from competing tags if single-frequency units are used). Researchers considering application of this technique should also attempt to design the receiver array so that the MCP described by their locations covers the maximum extent of the habitat to be studied while maintaining receiver coverage.

The agreement between the simulated shark track and the mean-position estimates indicates that this method can be used to determine the location of tagged sharks. However, the center-of-activity nature of the mean-position estimates makes direct comparison of these data problematic. The mean-position algorithm represents a position based on the location of the shark over the period Δt, whereas track data were point estimates. Thus, it is unlikely that there would be no differences between location estimates when these two data sets are compared.

The observation that distances between consecutive points from the mean-position algorithm were significantly lower than those from active tracking result from a number of factors. Firstly, because blacktip sharks were always moving, the position was an estimate of the mean for the period of Δt that is equivalent to a short-term center of activity (Hayne 1949). Thus the positions estimated by the algorithm will not accurately describe the full movement of the animal, especially when animals perform frequent turns as suggested by the active-tracking data. Secondly, the positions were limited to the area within the MCP described by the locations of the receivers. This meant that the sharks' movement would be underestimated if they swam outside of the MCP. Thirdly, the distances between successive positions of manually tracked animals was at times overestimated because of the distance between the vessel and the shark being based on the level of receiver gain at which the signal could no longer be detected. The implication of these results is that location data from the mean-position algorithm should not be used to study short-term behavior (e.g., swimming speed or fine-scale habitat utilization) but are better applied to studies that investigate long-term movement patterns, space utilization patterns over broader areas and home range.

The clumping of bearings between successive locations using the mean-position algorithm was a result of the arrangement of the receiver array and the number of receivers that logged a signal during each time period. In the situation where the location estimate is based on data from one or two receivers during a time period, the location will fall on either the receiver location or the line between two receivers. Because many of the locations were based on instances where only one or two receivers had logged a signal, these types of locations were common and so movements were most commonly recorded along these lines. Because most of these lines run parallel to the longitudinal axis of the bay, these angles (60–80° and 220–240°) were the most commonly recorded. As the number of instances with which locations were estimated was based on data from one and two receivers, the dominance of these angles decreased, and at Δt = 60 min, the distribution of bearings was uniform. The probability that a location estimate will be on the lines joining receiver locations is one reason that the distributions of bearings were different between the mean-position algorithm and active tracking. However, differences also occurred because

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of the center-of-activity nature of the mean-position algorithm.

There are a number of factors that limit the accuracy of the location estimates from the mean-position algorithm. Animals that swam outside of the MCP described by the receiver locations for extended periods could not be accurately located, and their position was estimated to be on the boundary of the MCP. However, location estimates for animals that made brief excursions outside of the MCP should not have been greatly impacted because of the center-of-activity nature of the technique. Interference with signals may also have reduced accuracy. Interference from physical conditions such a heavy rain, sea conditions, or boat traffic can reduce the reception range in the short term and so limit the number of receptions and number of receivers at which they are recorded. A second source of interference is overlapping signals from multiple tags in the same area. The tags used in this study used random repeat intervals (see Voegeli et al. 2001) to minimize this problem. However, early in the summer, the blacktip sharks spend most of their time in a small portion of northern Terra Ceia Bay (Heupel and Hueter 2001), making it possible that overlapping signals did cause interference.

All forms of telemetry have associated levels of accuracy and precision in relation to position estimates. Manual telemetry relies on the accuracy of the bearing from the vessels to the signal and the method of estimating the distance to the signal. Errors associated with this type of position estimation have rarely been addressed. For example, in a recent review of shark acoustic telemetry, Sundstrom et al. (2001) did not discuss the size of errors in position estimates or identify studies where they were. Terrestrial studies using radiotelemetry have considered error estimates to a greater extent than aquatic studies. For example, Schmutz and White (1990) examined error in the position estimates based on triangulation (a method often used in long-term studies where animals are relocated at regular intervals) and concluded that substantial errors occur owing to animal movement between triangulation fixes. In contrast to these manual telemetry methods, automated systems using signal reception time methods typically provide estimates of error associated with position estimates. This is partly because of the high level of accuracy and the ease of error estimation using fixed location tags (e.g., Smith et al. 1998). Estimates of position errors in these systems are typically between 1 and 2 m but increase when tags are located outside the baseline of the receivers (Bégout and Lagardère 1995; Cote et al. 1998; Voegeli et al. 2001). Thus the mean-position algorithm will not provide location estimates as accurate as the signal reception time method. The magnitude of errors relative to active-tracking techniques cannot be assessed without further investigation of potential errors in active tracking. However, by comparing the mean-position algorithm results to those from active tracking, the differences between these techniques can be assessed.

In designing a study of animal behavior using telemetry methods, the researcher must consider the requirements in terms of cost, manpower, spatial and temporal coverage, and positional accuracy. The mean-position technique demonstrated in this paper provides an alternative to labor-intensive active tracking and expensive linked acoustic telemetry systems. It allows a researcher to use data from relatively inexpensive, independent omnidirectional receivers arranged in an array to estimate the location of an animal and so study its behavior. This allows the researcher to obtain greater spatial coverage of a study site for long-term movement studies.

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Diurnal variation in bottom trawl survey catches: does it pay to adjust?

Vidar Hjellvik, Olav Rune Godø, and Dag Tjøstheim

Abstract: The diurnal bias of bottom trawl survey catches is studied with the purpose of adjusting for it and thereby improving the accuracy of abundance estimates. The correction term is estimated with uncertainty and thus increases the variance of the resulting abundance estimate. To investigate this adequately, we use a stochastic model describing diurnal fluctuations and examine the annual variation of the diurnal amplitude as a function of species and length. The diurnal amplitude is fairly stable for large fish, and for these, the bias-corrected estimate leads to a moderate increase in variance. For small fish, the diurnal amplitude is unstable, however, and the correction of diurnal bias occurs at the expense of a large increase in variance. This unstable amplitude also leads to a large year-to-year variation in catchability for small fish. For haddock (*Melanogrammus aeglefinus*), the diurnal amplitude depends heavily on fish length, indicating a strong decrease in catchability with decreasing fish length.

Résumé : Nous avons étudié l'erreur systématique reliée à la période de la journée dans les inventaires faits à partir de récoltes au chalut de fond dans le but de trouver un ajustement et ainsi améliorer les estimations d'abondance. L'estimation du terme de correction comporte de l'incertitude, ce qui augmente la variance des estimations d'abondance. Un modèle stochastique qui décrit les fluctuations diurnes et l'examen de la variation annuelle de l'amplitude diurne en fonction des espèces et des longueurs nous ont permis d'étudier ce problème adéquatement. L'amplitude diurne est assez stable chez les gros poissons et, pour eux, une estimation corrigée entraîne une augmentation modeste de la variance. Pour les petits poissons, cependant, l'amplitude diurne est instable et toute correction de l'erreur amène une importante augmentation de la variance. Cette amplitude instable cause aussi une grande variation inter-annuelle de la capturabilité des petits poissons. Chez l'Aiglefin (*Melanogrammus aeglefinus*), l'amplitude diurne est fortement reliée à la longueur des poissons, avec une forte décroissance de la capturabilité en fonction de la diminution de la longueur du poisson.

[Traduit par la Rédaction]

Introduction

A main motivation for studying diurnal oscillations, besides understanding them biologically, is to obtain more accurate abundance estimates. Efficiency of trawl surveys is dependent on the ability of the gear to catch the available fish and further, the availability of target species to the trawl (Godø 1994). Diurnal fluctuations may affect both of these factors, and if not adjusted for, diurnal oscillations could cause a large bias. Implicitly, the bias is included in the catchability coefficient when converting survey catches to abundance estimates. This coefficient is usually considered constant from one year to another but is well known to be subject to considerable variation (e.g., Pennington and Godø 1995). If the impacts of the diurnal fluctuations vary from year to year, this may serve to explain temporal variation in the catchability coefficient. A main goal of our paper is to examine the effect of diurnal bias on catchability, and its implications for survey abundance estimation.

To avoid diurnal bias, surveys are often conducted during the day only, under the assumption that the highest catches (usually during daytime) give the most representative picture of the stock (Wakabayashi et al. 1985). This leads to at least three problems. First, for some species nighttime catches are higher than daytime catches (Walsh 1988; Casey and Myers 1998). Second, it is not quite clear how daytime catches should be defined. As shown in Hjellvik et al. (2001), simply using the times of sunrise and sunset is not optimal in general (cf. also the problem of polar night in the Barents Sea). Third, there is a feeling that only using daytime measurements is tantamount to throwing away valuable information and not exploiting the measurements in an adequate way. Survey vessel time is expensive and optimal use of available resources is crucial for the quality of the survey results (Pennington and Vølstad 1991).

One might expect that including nighttime measurements will, if properly adjusted for diurnal bias, lead to more accurate abundance estimates. To our knowledge, this hypothesis has not been carefully examined. An important reason for this is the lack of an appropriate way of handling the associated uncertainty. A major objective of this paper is to show that the modeling technique of Hjellvik et al. (2001) can be used to approach this problem. The answer will depend critically on the annual stability of the diurnal variations. The bias adjustment depends on an uncertain estimate of the

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V. Hjellvik[1] **and O.R. Godø.** Institute of Marine Research, P.O. Box 1870, Nordnes, N-5817 Bergen, Norway.
D. Tjøstheim. Department of Mathematics, University of Bergen, Johs. Brunsgt 12, N-5008 Bergen, Norway.

[1]Corresponding author (e-mail: vidarh@imr.no).

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bias, and this added uncertainty is more serious if the diurnal fluctuations vary significantly from one year to another, because the bias then must be estimated separately each year and the added uncertainty may cancel the benefit of including nighttime measurements. Demer and Hewitt (1995) applied a temporal compensation function to adjust upward acoustic biomass estimates of Antarctic krill (*Euphausia superba*). In their modeling, they did not take into account the uncertainty involved in estimating the compensation function, but they found that the coefficient of variation in most cases increased after adjusting for diurnal variation, contrary to their expectation.

Demonstration of annual changes in diurnal variation in trawl catches is simultaneously a manifestation of varying catchability. Trawl survey catches are often one of the basic sources of information used in modeling ecosystem interactions and competition both between sizes of the same species and between different species. If catchability varies substantially in time and space, the traditional approach where catchability is considered constant (Godø 1994) might lead to misinterpretation of the dynamics of the ecosystem and hence reduce the quality of advanced modeling in stock assessment. In this paper, we demonstrate that it is possible to evaluate quantitatively changes in catchability based on the accumulated information collected during the surveys.

Material and methods

The data

Combined acoustic and bottom-trawl surveys for demersal fish have been conducted annually in the Barents Sea during winter (January–March) and in autumn (August–September) by The Institute of Marine Research, Bergen. We use the data from 1985 to 2000. For the winter survey, the time span does not vary much during the period (Fig. 1), but there have been some changes in the area covered. In 1993, the survey area was expanded northeastwards because it was clear by then that the small fish were not completely covered in the traditional survey area. In 1997 and 1998, the vessels were not allowed to enter the Russian zone, and thus the eastern part of the area was not covered. In 1999, the same area remained partially uncovered because of ice conditions. For the autumn survey, the time span varies considerably from year to year, and in 1995, the area covered was substantially expanded to the southeast in an attempt to cover the whole stock. Various adjustments in equipment have also taken place (Jakobsen et al. 1997). Two of the most important adjustments were the introduction of rockhopper gear in 1989 and a reduction in cod-end mesh size in 1994, and both strongly influenced the catch of small fish (<20 cm).

We started out with the length groups (length in centimetres) 0–10, 11–15, 16–22, 23–31, 32–44, 45–63, 64–90, and 90+, which are roughly the same as in Korsbrekke and Nakken (1999). However, we found it convenient to join the two first groups to a 0- to 15-cm group and the three last ones to a group of 45+ cm. The five resulting length groups roughly correspond to ages 1 through 4 and 5+ for cod.

We have considered six species: cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), deep-sea redfish (*Sebastes mentella*), Norway haddock (*Sebastes marinus*), Greenland halibut (*Reinhardtius hippoglossoides*), and long rough dab (*Hippoglossoides platessoides*). The number of species is much smaller than in Casey and Myers (1998) and the geographical region is more restricted, but the purpose of our study is rather different with the emphasis on adjusting for diurnal effects and the uncertainty involved in the adjustment.

Models

Technical details on models and methods are given in Hjellvik et al. (2001) and in Appendix A. Here we only give a brief summary. The basic observations are the number of fish n_i caught in the ith haul. However, the model treats log-transformed data

$$y_i = \log(n_i \cdot l_i^{-1} + 1) \doteq \log(x_i)$$

where l_i is the towed distance. We assume that the total variation in fish density is caused by a day-to-day variation, a superimposed diurnal variation, and random noise, so that

$$(1) \qquad y_i = \mu_{d(i)} + f(t_i) + \varepsilon_i$$

Here, t_i is the apparent local time of the ith haul (cf. Appendix A), f is a function describing the diurnal variation, and $\mu_{d(i)}$ is the daytime catch level on day $d(i)$ when haul i is taken. Finally, ε_i represents the random noise component.

The function f can be estimated nonparametrically without making any assumptions on its shape, or we can assume that it is given by a known parametric function for which parameters must be estimated.

We have found two parametric functions to be especially useful: the sinusoid

$$(2) \qquad f_S(t) = f_S(t;\, D) = (D/2)\sin((t - 6)\pi/12) - (D/2), \quad 0 \le t < 24$$

where $D/2$ is the amplitude; and the symmetric logistic function

$$(3) \qquad f_L(t) = f_L(t;\, D, \alpha, \beta) = \begin{cases} \dfrac{De^{\alpha(t-\beta)}}{1+e^{\alpha(t-\beta)}} - \dfrac{De^{\alpha(12-\beta)}}{1+e^{\alpha(12-\beta)}}, & 0 \le t \le 12 \\ \dfrac{De^{\alpha(24-t-\beta)}}{1+e^{\alpha(24-t-\beta)}} - \dfrac{De^{\alpha(12-\beta)}}{1+e^{\alpha(12-\beta)}}, & 12 < t < 24 \end{cases}$$

Both functions have been normalized so that their maximum is at $f(12) = 0$, which means that $\mu_{d(i)}$ can be interpreted as the expected value of y_i at day $d(i)$ at noon. The sinusoid function f_S represents a situation with smooth diurnal variations, whereas the logistic function f_L describes a scenario with an approximately constant night level, another constant day level, and a transition phase between them. The length and location of the transition phase are determined by the parameters α and β, respectively.

Note also that both eqs. 2 and 3 are linear in D. Thus, if α and β are kept fixed in eq. 3, then eq. 1 with $f = f_L$ is a linear model (see Appendix A for details). The main advantage of the linear approximation is that it is much faster, especially when bootstrapping (see the section Uncertainty estimates). If fixed values of α and β are used, we take $\alpha = 2$ (corre-

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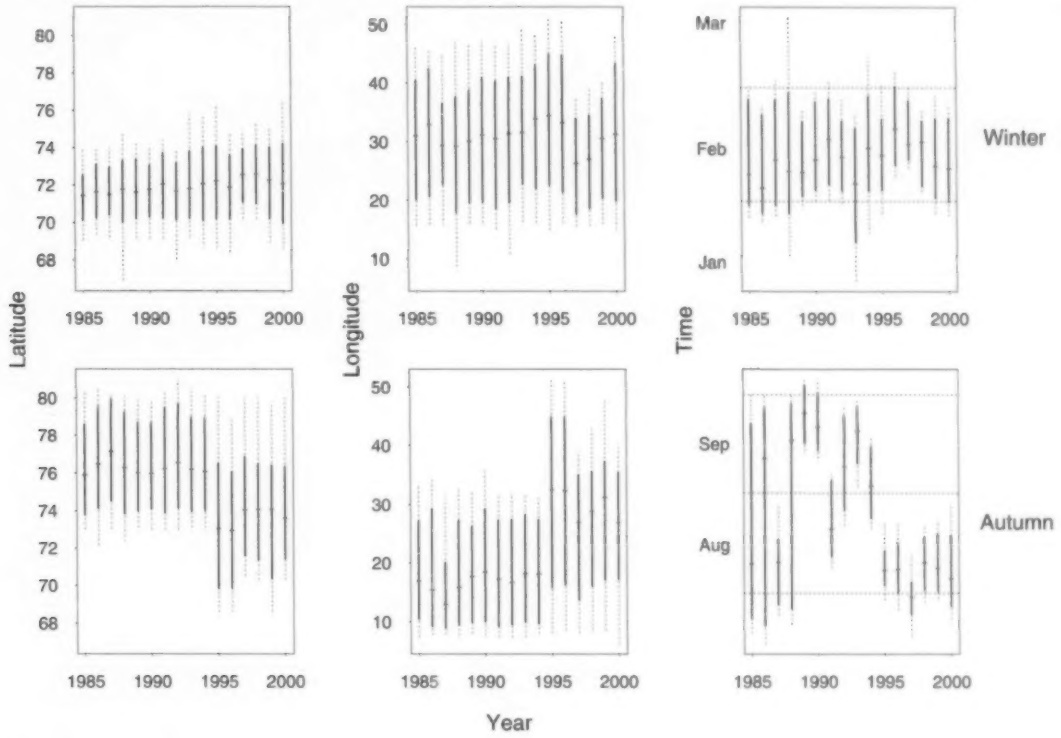
Fig. 1. Geographical extent and time span of the winter and autumn surveys. All stations were within the area or time indicated in the figure, and 80% were within the intervals indicated by solid lines. The medians are also given. Horizontal dotted lines indicate a change of month.



sponding to a transition phase of approximately 3 h) and β between 7 and 8. These values are based on our experience in Hjellvik et al. (2001) and on the computer runs in this paper.

The S-PLUS functions "nls" and "lm" (cf. Venables and Ripley 1997, sections 6 and 9) were used for estimating the parameters in the nonlinear and linear cases, respectively. The standard errors of the parameter estimates from these functions have been used to calculate confidence intervals.

Zero catches

In any study of diurnal variation, it is important to look carefully at zero catches. In Hjellvik et al. (2001), where the data were restricted to cod, it was found that the zero catches were roughly evenly distributed throughout the 24-h cycle and were therefore omitted. But clearly an uneven distribution of zero catches could be an added indication of diurnal effects, and omitting them would lead to an underestimation of the diurnal oscillations. Including all zero catches, as seems to be the case in some earlier studies, would also lead to an underestimation, because getting zero catches may simply mean that there are no fish of various length groups and species in certain locations. If included, the catches from such areas would contribute to the estimate of D just as much as do non-zero catches. Thus, if only zero catches are taken on a particular day, that day would draw the estimate of the diurnal amplitude D downwards. The same argument is valid for the simpler night–day level models used earlier. The zero catches taken outside the distribution area of a given length group and species should be excluded, and only those. In practice, this is not easy to achieve, but one possible strategy is to exclude all catches, zero and non-zero, taken on days with more than a certain percentage of zero catches, and include all other catches. Varying the threshold percentage yields a sensitivity study as to what degree the zero catches influence the results.

Adjusting for diurnal variation

When a functional relationship such as eq. 2 or eq. 3 is established, the catches can be adjusted accordingly to correct for diurnal bias. In a situation where f (f_L or f_S) gives a good fit and f has its maximum $f(t) = 0$ at $t = 12$, the natural adjustment of a measurement y_i taken at local time t_i is

$$y_{i,\text{adj}} = y_i - f(t_i) \quad (4)$$

where in practice f has to be replaced by its estimate $\hat{f}(t; D, \alpha, \beta) = f(t; \hat{D}, \hat{\alpha}, \hat{\beta})$.

We are ultimately interested in the influence of the adjustment on the abundance indices, but here we simplify by looking at the bias and uncertainty of the means $\bar{y}$ and $\bar{x}$. Alternatively, the medians $m(y)$ and $m(x)$ could be used. For the nonlogarithmic data, $m(x)$ is much more robust than $\bar{x}$ in terms of coefficient of variation, but smaller (if $y \sim N(\mu, \sigma^2)$ and $x = \exp(y)$, then $\text{mean}(x)/\text{median}(x) = \exp(\sigma^2/2)$). We have followed the traditional approach by using the mean.

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Uncertainty estimates

Calculating the uncertainty of abundance indices is not straightforward because of, among other things, the uneven geographical distribution and the movement of the fish. Ideally, the survey should be repeated under identical conditions. If such hypothetical surveys could be conducted, then for the kth survey we would have

$$(5) \qquad y_i^{(k)} = \mu_{d(i)} + f(t_i) + \varepsilon_i^{(k)}$$

with $\mu_{d(i)}$ and $f(t_i)$ being fixed because of the identical circumstances of the surveys. The mean and its uncertainty could then be computed by looking at $y_i^{(k)}$, $k = 1,2,\ldots$.

This is impossible in practice, but we can mimic the above situation by using the bootstrap technique. First we estimate $\mu_{d(i)}$ and $f(t_i)$ from the data from a particular survey to obtain $\hat{\mu}_{d(i)}$ and $\hat{f}(t_i)$. Then we calculate the estimated residuals

$$(6) \qquad \hat{\varepsilon}_i = y_i - \hat{\mu}_{d(i)} - \hat{f}(t_i)$$

The estimated random variation is now contained in $\{\hat{\varepsilon}_i\}$, and we can create new realisations $\hat{\varepsilon}_i^{(k)}$, or $\varepsilon_i^{*(k)}$ in bootstrap notation, by drawing random samples, with replacement, from $\{\hat{\varepsilon}_i\}$, creating bootstrap realisations

$$(7) \qquad y_i^{*(k)} = \hat{\mu}_{d(i)} + \hat{f}(t_i) + \varepsilon_i^{*(k)}$$

where $\hat{\mu}_{d(i)}$ and $\hat{f}(t_i)$ are kept fixed in accordance with eq. 5. For each realisation $\{y_i^{*(k)}\}$, we can estimate the mean $\bar{y}^{*(k)}$, and bootstrap confidence intervals can be formed from the empirical distribution of $\{\bar{y}^{*(k)}\}$.

To examine the effect of bias correction, we first go back to the idealized situation (eq. 5). Now f and $\mu_{d(i)}$ are unknown quantities that need to be estimated in order to correct for diurnal bias. We can use the nls/lm algorithm to obtain $\hat{f}^{(k)}$ (and $\hat{\mu}_{d(i)}^{(k)}$) for each realisation and then compute the adjustment

$$y_{i,\text{adj}}^{(k)} = y_i^{(k)} - \hat{f}^{(k)}(t_i)$$

and finally the mean and its uncertainty is found from $\{y_{i,\text{adj}}^{(k)}\}$. When employing the bootstrap, we use re-estimates $f^{*(k)}(t_i)$ (and $\hat{\mu}_{d(i)}^{*(k)}$) for each bootstrap realisation $\{y_i^{*(k)}\}$, with $y_i^{*(k)}$ given by eq. 7, and calculate

$$(8) \qquad y_{i,\text{adj}}^{*(k)} = y_i^{*(k)} - f^{*(k)}(t_i)$$

The fact that we have to estimate f means that although we correct for the bias, the added variability of the estimated f leads to larger variability in $y_{i,\text{adj}}$ than in y_i in eq. 4, which again leads to larger uncertainty in the adjusted abundance estimate. If f varies from one year to another, it has to be estimated separately for each year, which implies that there are fewer data points available for this estimation. This situation is represented by the bootstrap estimate in eq. 8, with $\{y_i\}$ being equal to the observed data points in each particular year. The effect on uncertainty is illustrated by a simple example given in Appendix A.

If, however, f is constant (i.e., the parameters α, β, and D are the same) from year to year, a more accurate estimate of f is obtained by pooling the data for the various years. As the number of available years increases, f can be considered to be known compared to the other unknown parameters $\mu_{d(i)}$, $i = 1,\ldots,n_d$. We can then use the estimate $\hat{f}$ (instead of $f^{*(k)}$) for all of the bootstrap realisations and the adjustment is given by

$$(9) \qquad y_{i,\text{adj}}^{*(k)} = y_i^{*(k)} - \hat{f}(t_i)$$

The resulting bootstrap confidence intervals correspond to a situation where f is known or approximately to a situation where f is stable from one year to another so that it can be estimated with high precision.

If diurnal fluctuations are ignored, or if only day catches are used, eq. 1 may be replaced by the simpler model

$$(10) \qquad y_i = \mu_{d(i)} + \varepsilon_i$$

An alternative, which is often used in practice, is to stratify geographically instead of by day.

Results

Zero catches

The effect of zero catches was examined by analysing the winter data for cod and haddock. For small- and medium-sized haddock, there was a clearly significant overweight of zero catches at night time (Fig. 2). In most other cases, the tendency was the same, but not so clear. For comparison, density estimates for simulated data sets of the same size but with the zero catch observations randomized over the 24-h cycle are included in the graphs. As will be seen, this is consistent with larger diurnal variation for small- and medium-sized haddock. Also note that zero catches constitute a much higher percentage of the total data material for haddock than for cod, even after the catches taken at days with only zero catches were eliminated. As expected, the results for haddock more than those for cod depend on the strategy for handling zero catches (Table 1). The average diurnal amplitude $\hat{D}$ varied when different strategies were applied, and including all zero catches yielded the smallest $\hat{D}$ in all cases as expected from the argument in the Zero catches section of Material and methods. On the other hand, there was no single strategy yielding the highest $\hat{D}$ in all cases, but setting the threshold at 50% non-zero catches seemed to yield overall reasonable results, and this strategy has been used in all subsequent calculations. Note that in Hjellvik et al. (2001) strategy *a* "exclude all" was employed, but it is seen that for cod this gives rather similar results to those obtained using the 50% strategy.

The shape of the diurnal variation

The nature and shape of the diurnal oscillations for winter cod data were analysed quite extensively in section 2 of Hjellvik et al. (2001). Nonparametric estimates for the species considered in this paper are depicted in Fig. 3. The S-PLUS function smooth.spline (see Venables and Ripley 1997) was used here and elsewhere for nonparametric estimates. We carried through a successive sequence of tests, as in Hjellvik et al. (2001), of the hypotheses $f = C$ (constant), $f = f_S$, and $f = f_L$ (see Appendix A for details) and found that the winter data are best described by f_L and the autumn data by f_S (Fig. 3). These are adopted as our standard in the fol-

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Fig. 2. Histograms and scaled density estimates (thick lines) for the time points at which zero catches were taken during days with at least one nonzero catch. The number of zero catches and their percentage of the total number of hauls are given by n and p, respectively. The dotted lines show density estimates for 50 data sets of size n simulated from a uniform (0,24) distribution. The S-PLUS function density was used for density estimates.

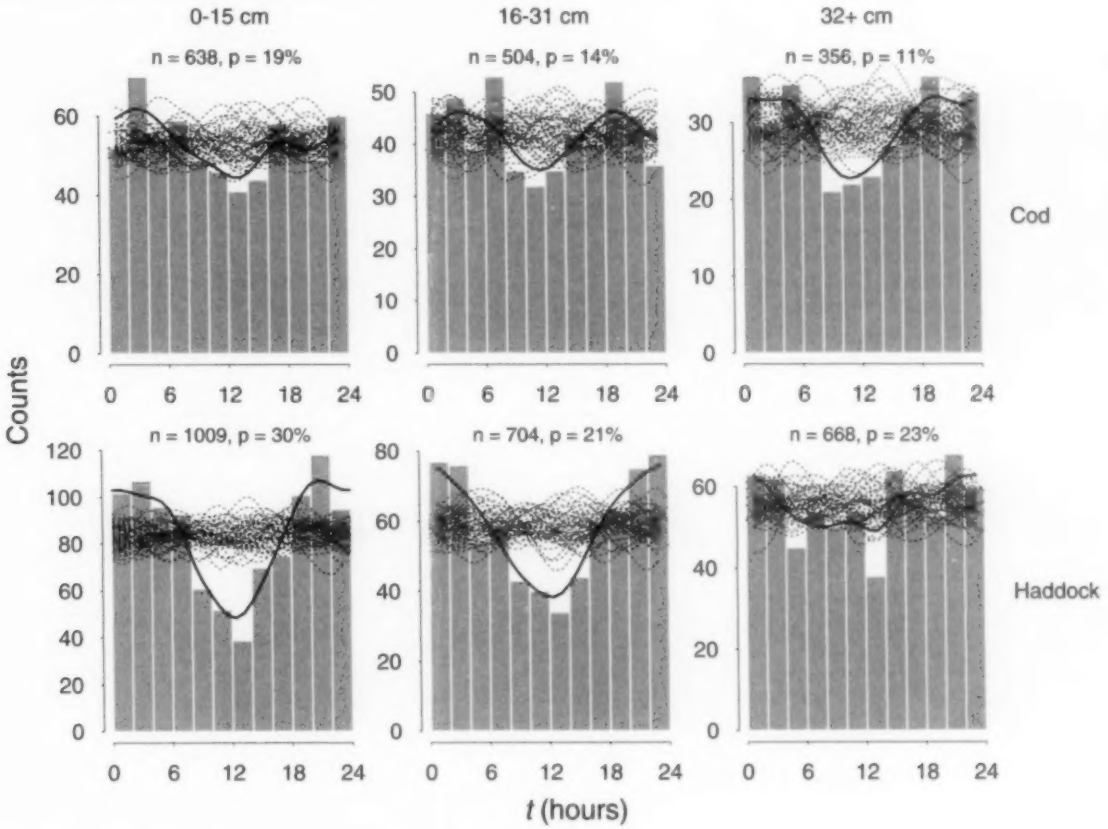

Table 1. Estimates of D in eq. 3 with $\alpha = 2$ and $\beta = 8$ when various strategies for handling zero catches are applied.

Species	Method	0–15 cm	16–31 cm	32+ cm
Cod	(*a*) exclude all	0.74 (0.049)	0.62 (0.042)	0.39 (0.042)
	(*b*) include all	0.57 (0.040)	0.58 (0.042)	0.38 (0.043)
	(*c*) include 1	0.64 (0.045)	0.59 (0.043)	0.42 (0.048)
	(*d*) include 25%	0.70 (0.049)	0.60 (0.043)	0.44 (0.049)
	(*e*) include 50%	0.74 (0.052)	0.63 (0.045)	0.45 (0.049)
	(*f*) include 75%	0.83 (0.056)	0.73 (0.046)	0.42 (0.047)
	(*g*) include 100%	0.89 (0.058)	0.70 (0.048)	0.39 (0.043)
Haddock	(*a*) exclude all	1.36 (0.060)	1.09 (0.060)	0.30 (0.062)
	(*b*) include all	1.21 (0.048)	1.06 (0.053)	0.26 (0.047)
	(*c*) include 1	1.33 (0.052)	1.18 (0.058)	0.33 (0.060)
	(*d*) include 25%	1.43 (0.056)	1.24 (0.061)	0.37 (0.064)
	(*e*) include 50%	1.59 (0.064)	1.31 (0.065)	0.45 (0.070)
	(*f*) include 75%	1.66 (0.074)	1.29 (0.070)	0.48 (0.074)
	(*g*) include 100%	1.65 (0.082)	1.25 (0.072)	0.38 (0.073)

Note: Standard errors are given in parentheses. (*a*) and (*b*), all zero catches are excluded or included; (*c*), all catches taken on days with at least one non-zero catch are included, all other catches are excluded; (*d*–*g*), all catches taken on days with at least 25, 50, 75, and 100% non-zero catches are included, all other catches are excluded.

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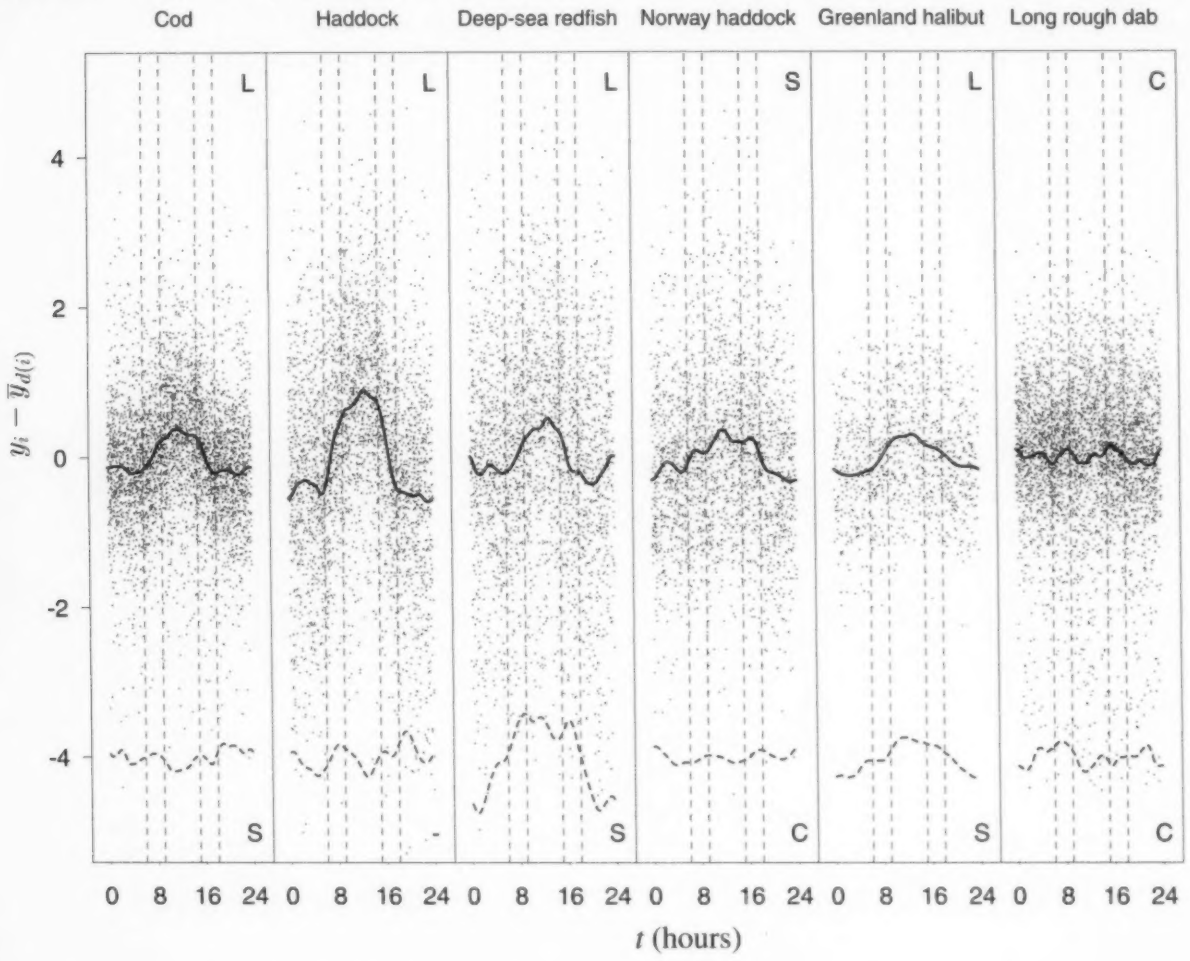
Fig. 3. Dots represent mean-adjusted catches $\{y_i - \bar{y}_{d(i)}\}$ from the winter surveys 1985–2000 for cod, haddock, deep-sea redfish, Norway haddock, Greenland halibut, and long rough dab. Solid curves represent the corresponding nonparametric estimates of $f(t)$. Broken curves represent the nonparametric estimates of $f(t)$ for the autumn data, adjusted downwards by 4 units. Vertical broken lines are drawn at t = 6, 9, 15, and 18. Results from tests of the hypotheses $f = C$, $f = f_S$, and $f = f_L$ are given in the upper (winter) and lower (autumn) right corners. C indicates that $f = C$ was not rejected at the 5% level. S indicates that $f = C$ was rejected, whereas $f = f_S$ was not. L indicates that $f = C$ and $f = f_S$ were rejected, whereas $f = f_L$ was not. A minus indicates that all three hypotheses were rejected.



lowing with f_L fitted to the winter data and f_S to the autumn data. Comparing f_L with nonparametric estimates for the length stratified winter data, there is generally a quite good agreement (Fig. 4). In particular, the choice of $\alpha = 2$ in eq. 3 seems appropriate.

Yearly estimates of diurnal variation

Motivated by the problem of obtaining adequate measures of uncertainty outlined in the Uncertainty estimates section, we were interested in detecting possible year-to-year differences in diurnal oscillations. We did this by fitting model 2 for the autumn data and model 3 (or, more precisely, model A3 in Appendix A) for the winter data for each year.

For small cod, some of the last years differ markedly from other years (Fig. 5), and the same is true to some extent for small haddock. For large cod and haddock and for other species, annual differences are smaller and can be ascribed to random fluctuations in many cases. We applied Simes-modified Bonferroni test (see Appendix A for details) to test the null hypothesis of no year-to-year differences in D for each combination of species and length group. The p values are given on the figure. At a 5% level, the null hypothesis was rejected in 8 of 10 cases for the two smallest length groups but only in 1 of 15 cases for the three largest length groups.

For the autumn data, there were relatively few cases with significant diurnal variation for other species than deep-sea redfish (Fig. 6). One exception is 1994 when D was significantly negative for all length groups for cod and long rough dab. As regards year-to-year differences in D, these were significant for all length groups of deep-sea redfish and long rough dab and for two length groups of cod. However, re-

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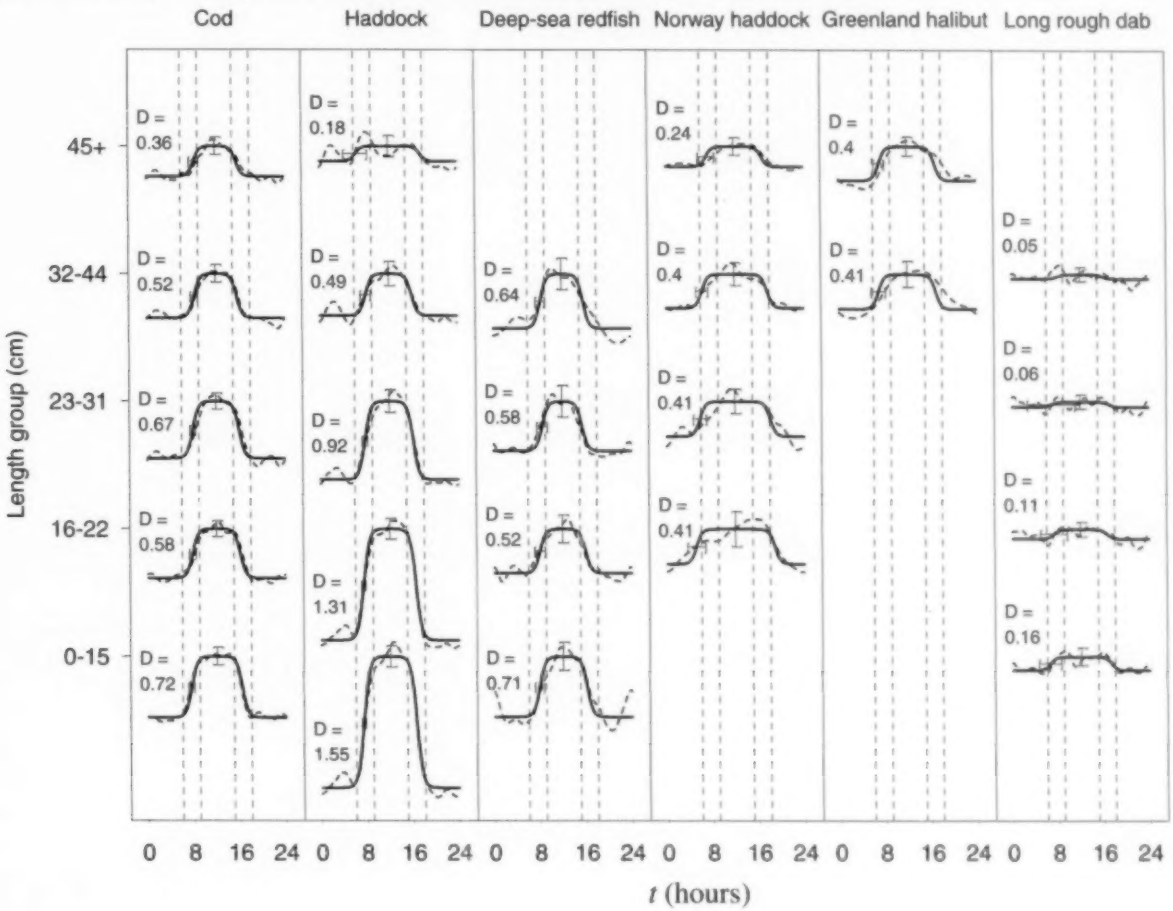
Fig. 4. Solid curves represent estimates of $f_L(t)$ (α = 2 kept fixed) for length-stratified data from the winter surveys 1985–2000 for cod, haddock, deep-sea redfish, Norway haddock, Greenland halibut, and long rough dab. Broken curves represent the corresponding nonparametric estimates of $f(t)$. Some length groups were excluded for some species because of few observations. Confidence intervals (95%) for D and β are indicated on the figure. Vertical broken lines are drawn at t = 6, 9, 15, and 18.



moving the estimates for 1994 for cod and long rough dab, year-to-year differences remained significant only for two length groups of long rough dab (Fig. 6).

Adjusting for diurnal effects

The variation in the diurnal amplitude D from one year to another contributes to the uncertainty in the abundance estimates as measured by the mean $\bar{y}$ of $\{y_i\}$. In this section, we show the results of applying the bias correction described in eq. 4 with accompanying bootstrapped confidence intervals based on eqs. 7–9, including the possibility of a varying D. Only the results for small cod and haddock (0–15 cm) will be displayed. For this length group, the diurnal variations are substantial and undergo significant yearly changes. All results in this section are based on 1000 bootstrap replicas.

The average catches $\bar{y}$ are represented by the point estimates marked as 1 in Fig. 7, which displays 6 years in which D values range from small to large. The corresponding 90% confidence intervals are obtained by bootstrapping as in formula 7. The bias-adjusted estimates are marked as 2 in Fig. 7, and as can be seen, the effect of the adjustment is quite dramatic when D is large. The adjustment formula (eq. 4) has been used, and bootstrap confidence intervals have been computed from $\{y_{i,\mathrm{adj}}^{*(k)}\}$ in eq. 8 based on yearly estimates of D. The adjusted intervals are presumably correctly located, but their width has increased because of the added uncertainty in estimating D. Actually, comparing with the day-only estimates in 4 and 5 in Fig. 7, the effect of the larger number of observations is more or less cancelled by the added uncertainty of the bias correction. In view of the section Uncertainty estimates (cf. also the example in Appendix A), this is exactly what one would expect. For species and length groups with a stable D (as judged, for example, by using tests depicted in Figs. 5 and 6), however, a more precise estimate of D can be obtained. As more and more data are accumulated, the uncertainty in D can be ignored compared with the uncertainty in $\bar{y}$, and the bootstrap confidence intervals based on a fixed $\hat{f}$ as in eq. 9 (corresponding to f known) can be used. This is not justified for small cod, but to give an impression of the size of the uncertainty re-

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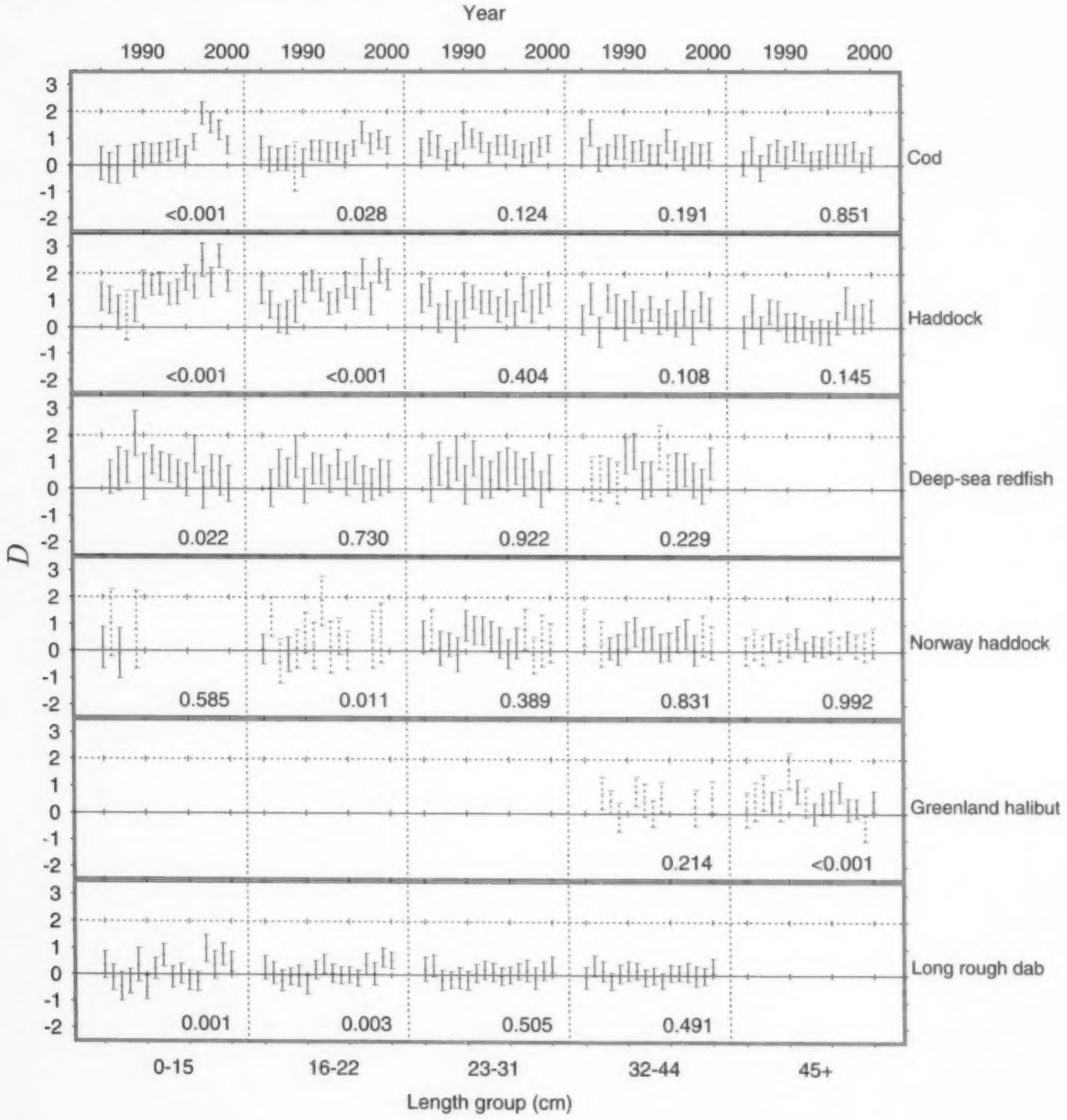
Fig. 5. Yearly estimates of D with 95% confidence intervals for the winter data 1985–2000. Years with $n - n_d - 1 < 20$ are excluded, and where $n - n_d - 1 < 50$, dotted lines are used. Here, n is the number of hauls and n_d the number of days. Model A3 has been used. The numbers at the bottom of each panel are p values for the observed $\hat{D}$s under the null hypothesis of no year-to-year differences in D for each combination of species and length group.



duction involved, these intervals have been included in Fig. 7, marked as 3. As anticipated, they have the same location as those adjusted with f unknown, and their widths are essentially the same as for the unadjusted intervals. In practice, with a moderate number of years available, there will be some uncertainty connected with the estimate of f even though it is based on data from all years. One should then apply the bootstrap algorithm in the section Uncertainty estimates on the data set consisting of observations from all years, and the resulting confidence intervals would be somewhat broader. We can check the location by computing the intervals using daytime measurements only. This was done by fitting and bootstrapping model 10, but in the bootstrap, we used the residuals (eq. 6) from the full model. A catch is

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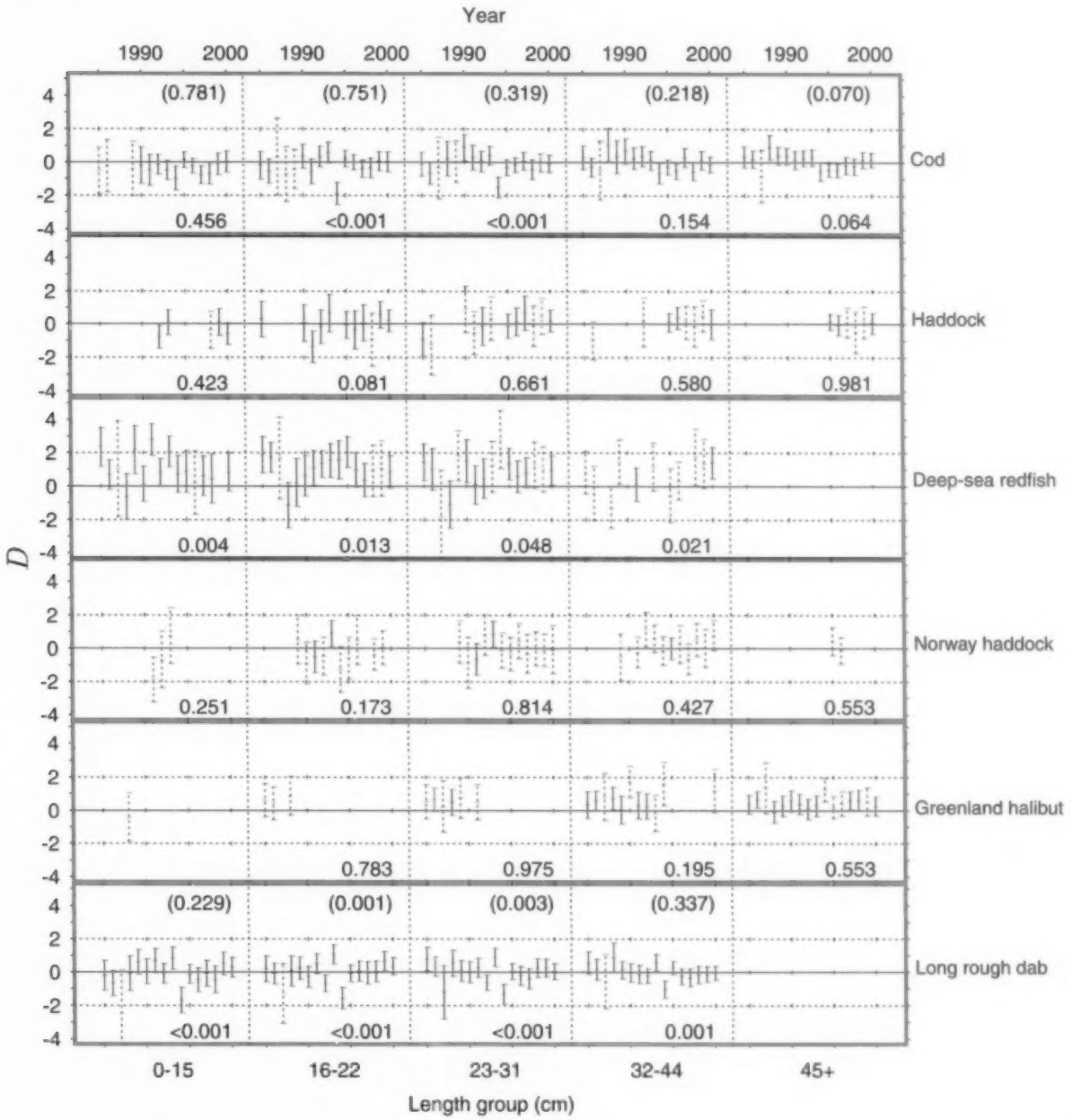
Fig. 6. Yearly estimates of D with 95% confidence intervals for the autumn data 1985–2000. Years with $n - n_d - 1 < 20$ are excluded, and where $n - n_d - 1 < 50$, dotted lines are used. Here, n is the number of hauls and n_d the number of days. Model 2 has been used. The numbers at the bottom of each panel are p values for the observed $\hat{D}$s under the null hypothesis of no year-to-year differences in D for each combination of species and length group. The numbers in parentheses for cod and long rough dab are the p values when 1994 is left out.



considered a daytime measurement if it is taken at time $\beta' \leq t_i \leq 24 - \beta'$, where $\beta' = 7.60$ is the estimate of β from the fit of eq. A3 in Appendix A. These intervals are marked as 4 in Fig. 7. Both location and width are about the same as for the bias-adjusted intervals obtained by using both night and day measurements, but with a tendency of the day intervals to be biased downwards. This is because day time is defined so that half of the transition phase between day and night is included. Defining day and night in terms of the altitude of the sun also yields very similar results, as can be seen from the intervals marked as 5 in Fig. 7. Here, based on Hjellvik et al. (2001), the catches taken when the sun is less than 5° un-

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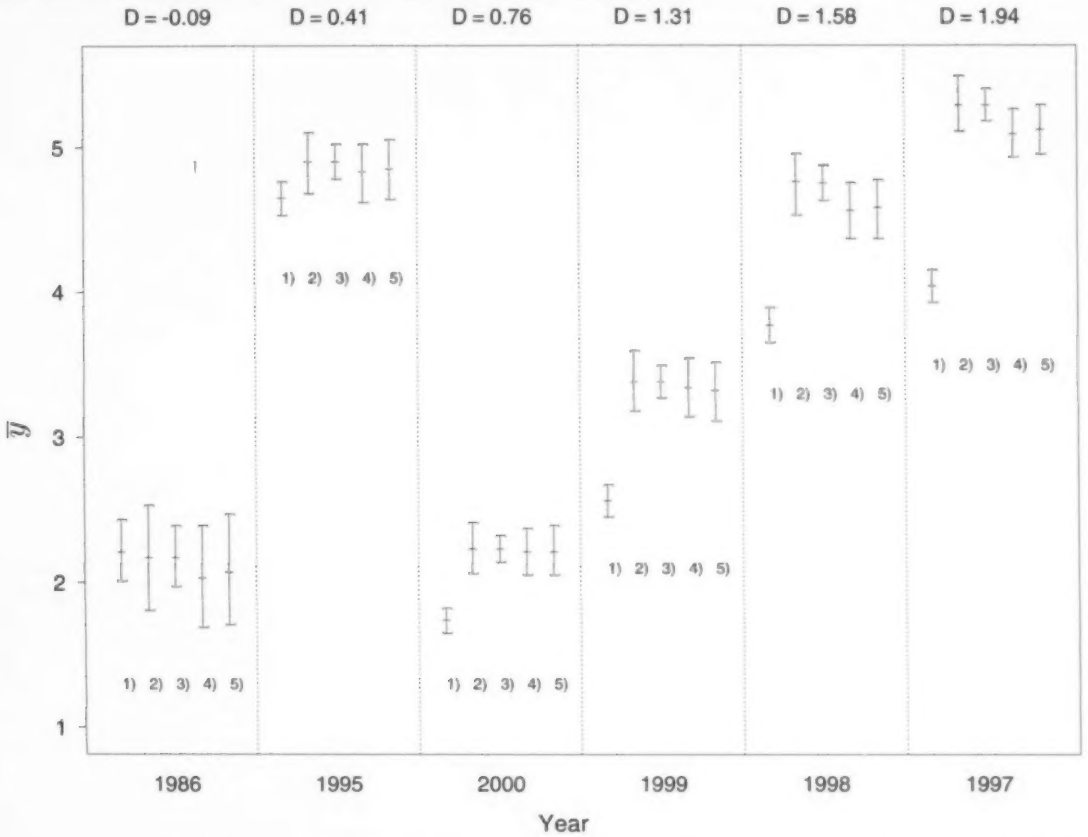
Fig. 7. Bootstrapped means and 90% confidence intervals for mean catches $\bar{y}$ for 0- to 15-cm cod, winter. From left to right: 1) unadjusted catches; 2) adjusted catches; 3) adjusted catches with f assumed known; 4) day catches with day defined as $\beta' \leq t_i \leq 24 - \beta$, where $\beta = 7.60$; and 5) day catches with day defined as the altitude of the sun being $\geq -5°$. The model $f_L(t)$ with $\alpha = 2$ and $\beta = 7.60$ fixed has been used in eqs. 8 and 9, and 1000 bootstrap replicas were used everywhere.



der the horizon are included (cf. also Korsbrekke and Nakken (1999)).

For the raw data, i.e., $x_i = e^{y_i}$, the mean for the adjusted catches of 0- to 15-cm cod is more than twice as high as the mean of the unadjusted catches in 1997–1999 when the diurnal variation is highest (Fig. 8). But that is also true for the standard deviation in both 1997 and 1998. The bootstrapped confidence intervals on the figure are centred at the observed means. The mean square error $\mathrm{MSE}(\bar{x}) = \mathrm{var}(\bar{x}) + \mathrm{bias}^2(\bar{x})$ was calculated by setting $\mathrm{var}(\bar{x})$ equal to the bootstrap variance of $\bar{x}$, and $\mathrm{bias}(\bar{x}) = \bar{x}_{\mathrm{adj}} - \bar{x}$. Similarly, $\mathrm{MSE}(\bar{x}_{\mathrm{adj}})$ was calculated by setting $\mathrm{bias}(\bar{x}_{\mathrm{adj}}) = 0$. The MSE was smaller for the adjusted means than for the unadjusted means in all cases for both cod and haddock (Fig. 8). For haddock, the unadjusted curve reveals a zigzag structure when plotted against year (Fig. 8*b*). This is amplified in the adjusted curve because the highest values of $\hat{D}$ occur in the years with the highest catch.

The significant annual variation in D for small cod and haddock also leads to huge year-to-year differences in the adjustment factor $\Delta_Q = \bar{x}_{\mathrm{adj}}/\bar{x}$ (Fig. 9). The year-to-year variation in Δ_Q tends to decrease with fish length, especially for haddock. Taking the uncertainty of the estimates into account, it is seen that as a rough approximation, Δ_Q for large fish can be taken to be constant. If the years 1997–1999 are excluded, Δ_Q also is much more stable for small cod. For haddock, the average Δ_Q is clearly decreasing with increasing fish length, whereas for cod, it is relatively independent of fish length, but somewhat larger for small fish.

Discussion

The main objective of this paper has been to study diurnal bias, to correct for it, and to examine its influence on the uncertainty of abundance estimates. The bias has been studied as a function of species, length group, and season. Particular emphasis was put on investigating its stability, or lack of such, from one year to another. The uncertainty of a bias-adjusted abundance estimate depends critically on that stability. In the following, we will discuss these aspects, starting with a short discussion of the model.

Some comments on the model

Our modeling approach is completely general and is applicable to any bias adjustment and uncertainty analysis of this kind. In particular, we believe that it may be useful in a

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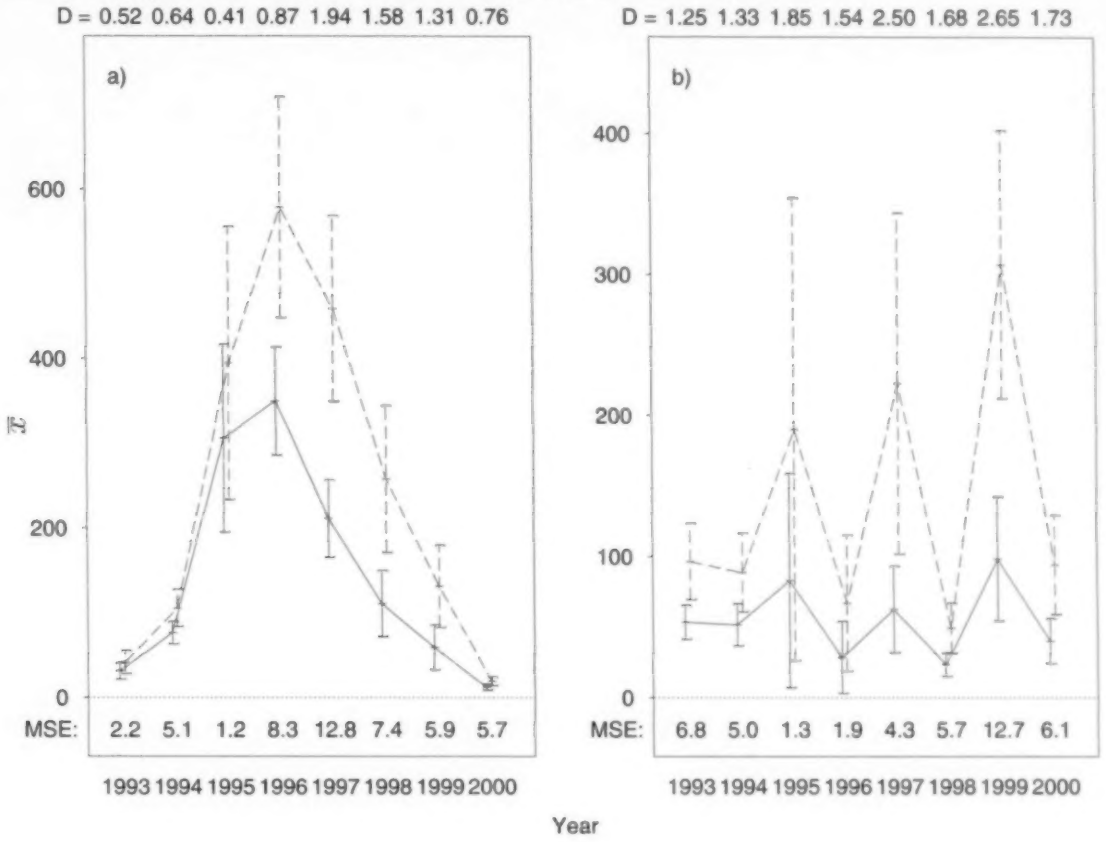
Fig. 8. Observed values of $\bar{x}$ (solid lines) and $\bar{x}_{adj}$ (dotted lines) for (*a*) 0- to 15-cm cod, winter, and (*b*) 0- to 15-cm haddock, with bootstrapped 90% confidence intervals. Unadjusted and adjusted estimates correspond to 1) and 2) in Fig. 7. For each year, $\hat{D}$ and the ratio MSE($\bar{x}$)/MSE($\bar{x}_{adj}$) are given at top and bottom, respectively, where MSE is mean square error.



combined study of acoustic surveys and trawl catches. The logistic model f_L with its smooth transition between night and day level is a refinement compared with the models of only two levels used by Casey and Myers (1998) and Korsbrekke and Nakken (1999). Casey and Myers (1998) base their classification on local times of sunrise and sunset, but to remove transitory effects, they omit hauls within 1 h of sunset or sunrise. Korsbrekke and Nakken (1999) use the altitude of the sun, and catches taken when the sun is less than 5° below the horizon are classified as daytime catches, which in view of the results of Hjellvik et al. (2001) seems sensible.

Still, our model is quite crude for several reasons. First, the function f is taken to be independent of the time of sunrise and sunset of day $d(i)$. Second, explanatory variables such as location and depth have been omitted. Third, species are interacting in an ecosystem, and the true dynamics and variation in abundance and composition cannot be fully explained merely from a simple single-species approach (Bogstad and Mehl 1997). For instance, can the larger diurnal variation for small fish be better understood in a multispecies context? The first point is discussed in Hjellvik et al. (2001), and a model containing explanatory variables and a preliminary investigation of the multispecies aspect are parts of the effort in Hjellvik et al. (unpublished data).

The assumption of independent, identically distributed residuals is not quite fulfilled, as there is a tendency of getting large residuals ε_i at stations with low predicted values. When taking antilogarithms, biased bootstrap distributions of $\bar{x}$ and $\bar{x}_{adj}$ result because large residuals ε_i^* in combination with large predicted values $\hat{y}_i^*$ may yield very large values of $x_i^* = \exp(y_i^*) = \exp(\varepsilon_i^* + \hat{y}_i^*) = \exp(\varepsilon_i^*)\exp(\hat{y}_i^*)$. Thus, the means of the bootstrap distributions are typically higher than the corresponding observed means. We have chosen to centre the confidence intervals so that they are symmetric around the observed means. In most cases, the largest residuals occur at nighttime (Fig. 10), but bootstrapping night and day data separately did not have much effect on the skewness of the confidence intervals. However, this approach typically produced slightly narrower confidence intervals. For the unadjusted means for haddock in 1995 and 1996, where the tendency of getting largest residuals at night is strongest, the variance was reduced to about 50%, but the ratio MSE($\bar{x}$)/MSE($\bar{x}_{adj}$) remained practically unchanged.

As a technical detail, it should be noted that for small values of α (slow transition), the parameter D is no longer

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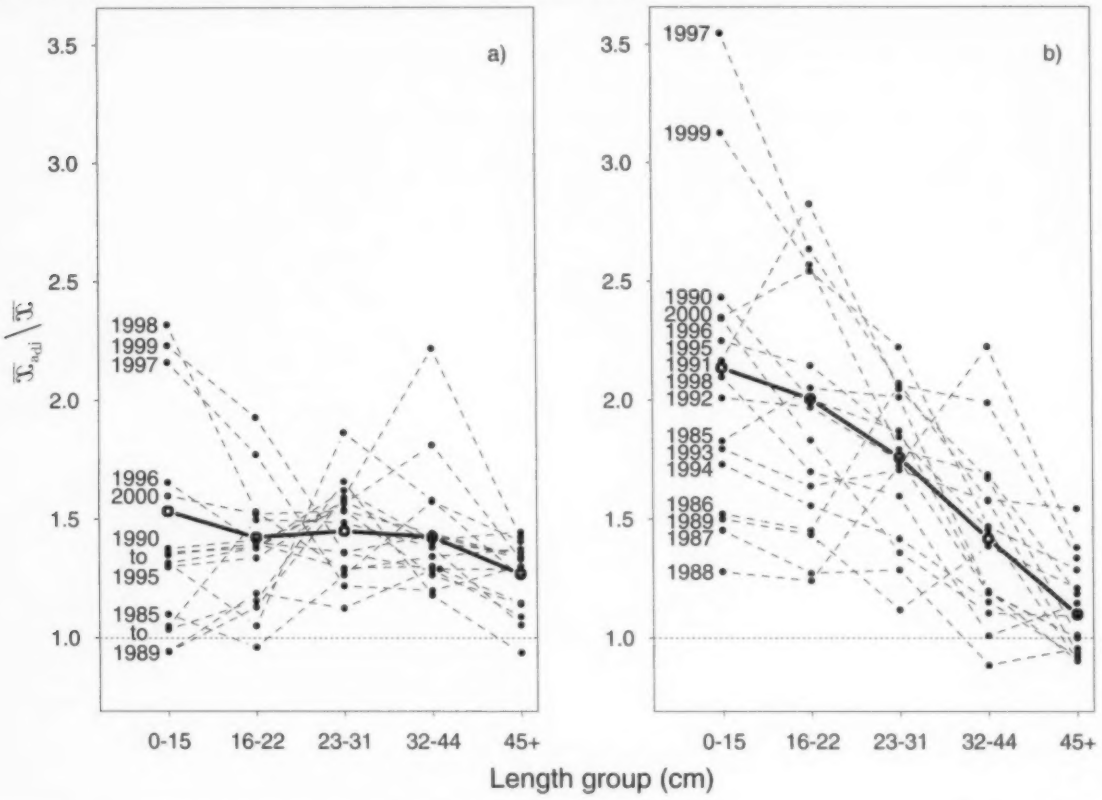
Fig. 9. The catchability ratio $\Delta_Q = \bar{x}_{adj}/\bar{x}$ for (*a*) cod, winter, and (*b*) haddock plotted against length for the years 1985–2000 (dotted lines). The thick lines are the averages of the dotted lines, weighted with the yearly number of hauls used.



equivalent to the difference between day and night levels. This is because the maximum and (or) minimum of $e^{\alpha(t-\beta)}/(1 + e^{\alpha(t-\beta)})$ in eq. 3 is not obtained for $0 \le t \le 12$. In such cases, the sinusoidal model f_S is a better choice, and in the case of small oscillations, when the estimation of α is encumbered with large uncertainty, we recommend that it be kept fixed, as has been done in most of this paper.

Instability of diurnal variation in time and dependence on depth and length

Relatively large seasonal differences were found in both amplitude and shape of diurnal oscillations. This is not surprising in view of the different light conditions, but it does not really create much trouble in the adjustment procedure as the most appropriate of the sinusoid and the logistic function can be applied in eq. 4. More serious from an assessment point of view is the instability of diurnal amplitude from one year to the next. The year-to-year differences are largest for small fish, in particular for small cod and haddock. There are no commercial data available for recruiting age groups, and thus survey-based assessment of these age groups is particularly important. To understand these fluctuations in D, it should be remembered that juvenile groundfish often have a pelagic distribution and therefore would be partly unavailable to the bottom trawl during their 1st year of life. Age-1 fish (corresponding more or less to the 0- to 15-cm length group) have not always completed bottom settlement at the time of the winter survey (Godø and Sunnanå 1992), and hence there will be a year effect on the availability of these groups to the trawl. Also, as shown by Aglen et al. (1999), small fish may have substantial vertical migration dynamics.

We know also that larger-sized cod migrate vertically, but the results of the present paper and new information from data storage tags indicate that the diurnal pattern is not as pronounced as for small fish (Godø and Michalsen 2000). Further, the substantial vertical herding (fish swimming from the safe pelagic zone into the catching volume of the trawl) observed for large fish (e.g., Ona and Godø 1990) will probably reduce the effect of diurnal migrations on catchability for these size groups.

This difference in behaviour is enhanced with increasing depth (Hjellvik et al., unpublished data), a larger depth generally leading to larger diurnal oscillations for small fish. Annual differences in average depth may therefore result in different D values. In fact, for small cod, there is a strong positive relationship ($R^2 = 0.84$) between average depth and $\hat{D}$ (Fig. 11). In particular, the high D values for 1997 and 1998 can be explained from this regression, as the winter survey did not enter the Russian zone during those years and the excluded eastern part is relatively shallow leading to a substantially larger average depth. The variation in average depth for the remaining years is minor and is caused partly by small year-to-year differences in the area covered (e.g.,

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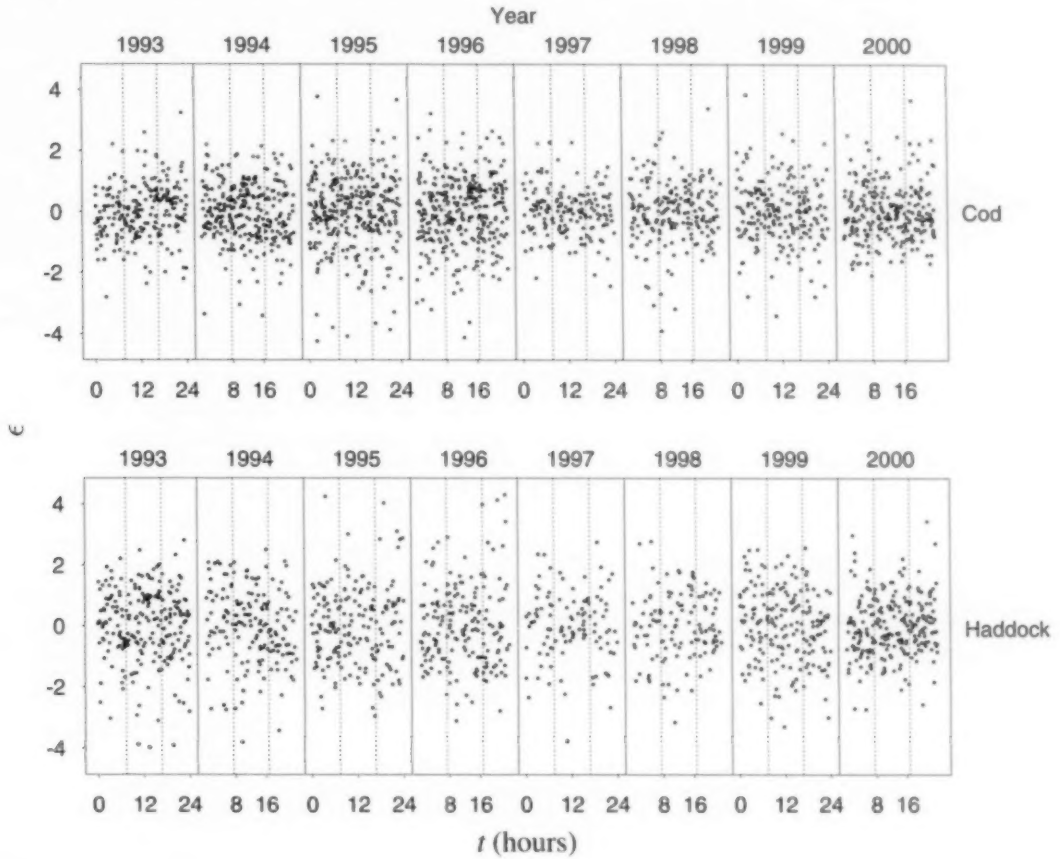
Fig. 10. Residuals from model 1 with $f = f_L$ plotted against time of day for 0- to 15-cm cod and haddock, winter. The model was fitted to data from each year separately, with $\alpha = 2$ and $\beta = 7.60$ (cod) or 7.34 (haddock) used in eq. 3. Dotted lines are drawn at $t = \beta$ and $t = 24 - \beta$.



resulting from varying ice conditions and changes in survey strategy), partly by year-to-year variation in the spatial distribution of small cod combined with our strategy for discarding of zero-catch hauls.

For small haddock, the annual dependence of $\hat{D}$ on depth as measured by R^2 is much weaker ($R^2 = 0.15$). However, this does not mean that the depth dependency as such is weaker for haddock than for cod. Because of the more restricted distribution of haddock, particularly towards the east (see Bergstad et al. 1987), the average depth was not affected in the same way by restrictions in area coverage in the Russian zone. The resulting small year-to-year variation in average depth explains the low R^2 value. Changes in fish density as measured by $\bar{y}$ explain more of the variation in $\hat{D}$ ($R^2 = 0.45$).

The transition around 1990 from low to higher values of D for small cod and haddock could be due to the change of ground gear in 1989 (cf. Engås and Godø 1989; Jakobsen et al. 1997), but a similar transition is not observed for other species.

Clearly, the temporal instability of diurnal variation needs to be more fully investigated, for example, by integrating results from hydroacoustics and multispecies effects. Especially, it would be of interest to examine the effect of bottom settlement on diurnal variation for small fish.

Catchability

The results of this paper show that the diurnal correction, as measured by the adjustment factor $\Delta_Q = \bar{x}_{adj}/\bar{x}$, in many cases exceeds a factor of 2. This implies that a sizable proportion of the catchability coefficient is made up of diurnal bias. Thus, the temporal instability of the adjustment factor indicates that there is a corresponding temporal instability of the catchability coefficient. Similarly, variation in Δ_Q with fish length indicates a length-dependent catchability coefficient. For cod, the dependency of Δ_Q on fish length found in this paper is not in complete agreement with the results in fig. 3 of Korsbrekke and Nakken (1999). They give the average day–night ratios for abundance indices for the years 1989–1996, and the highest ratio is obtained for 23- to 31-cm fish. However, using data from 1989–1996 only, our results are more similar to theirs with the highest Δ_Q obtained for 23- to 31-cm and 32- to 44-cm fish.

If other factors that make up the catchability coefficient are length independent, our results mean that the catchability for haddock (averaged over night and day) is clearly de-

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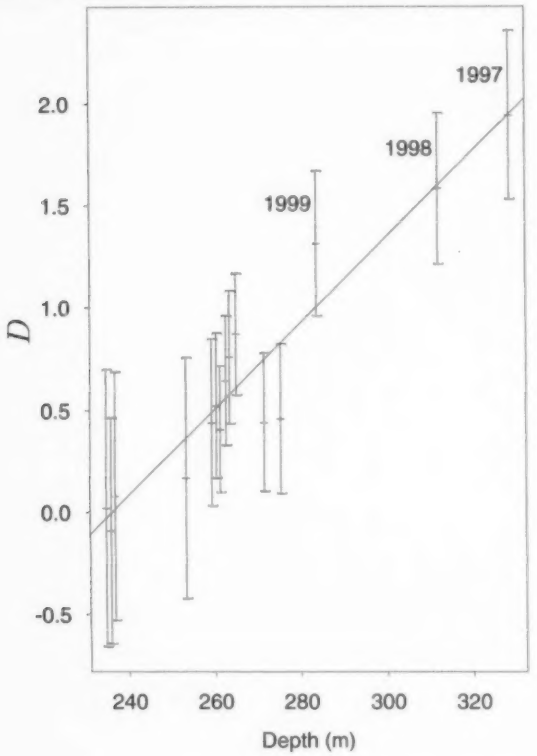
Fig. 11. Yearly estimates of D for 0- to 15-cm cod, winter, plotted against average yearly depth for the stations used for the estimates. Confidence intervals (95%) for D are included.



creasing with decreasing fish length in that night catches for small fish are much lower than day catches. Using a constant catchability, as is now done, would tend to underestimate the relative proportion of small fish by a factor that can be numerically derived from Δ_Q.

Does it pay to adjust?

We have argued for a model-based adjustment of survey trawl catches to minimise bias and variation caused by diurnal effects. In essence, the goal has been to examine integrity, within and between years, in the assessment results for individual species. Adjustment removes diurnal bias, but at the cost of increased uncertainty of the adjusted estimates. Thus, it is not obvious whether it pays to adjust. In fact, the answer to this question depends on our goal. Do we want to estimate absolute fish density? Are we only interested in tracking the changes in relative fish density for the different age or length groups over years? Or do we want to track each year-class over its life span?

Sometimes, in situations where the bias may be reduced at the cost of an increased variance, decision rules based on the MSE are used. Munro (1998) presents such a rule for a somewhat analogous situation. Our bootstrap method yields estimates of the variance of both the adjusted and unadjusted estimates and of the bias of the unadjusted estimates relative to the adjusted ones. But in terms of absolute abundance measures, the adjusted estimates are most likely biased downwards as well. Considering the winter data from 1993 through 2000 for small cod and haddock and first assuming unbiased adjusted estimates, the MSE is much smaller for the adjusted means than for the unadjusted ones. If the downward bias of the adjusted estimates (relative to absolute densities) is taken into account, the difference in MSE is enhanced. In terms of the MSE, adjustment seems to pay even for relatively small diurnal fluctuations (in 1995, with $D = 0.41$ for small cod, the MSE was about 15% larger for the unadjusted data than for the adjusted ones). In a situation where diurnal fluctuations are stable from year to year, the variance, and hence the MSE, of the adjusted means will be even smaller compared with the unadjusted ones, and adjustment will pay for even smaller values of D.

If the only issue of interest is to track relative changes in density of a given age or length group over years and there are no significant year-to-year differences in diurnal amplitude, then the adjustment will just introduce an extra source of variation and is not recommended. On the other hand, if there are substantial year-to-year differences, adjustment is generally recommended to remove year-dependent biases. One could use the modified Bonferroni test in Appendix A or some other test to check whether such differences exist. If one is interested in tracking year-classes from birth to extinction, one should adjust if there are substantial differences in diurnal variation between length groups.

In the above discussion, it has been assumed that catchability at day time is more or less stable over years. However, year-to-year differences in bottom settlement for small fish (e.g., Godø and Sunnanå 1992) may affect both daytime catchability and diurnal variation, and ideally the bottom settlement process should be monitored to control its effect on the catchability of small fish. In fact, adjustment may potentially reduce the quality of the time series of abundance estimates of age-1 fish if bottom settlement varies substantially from one year to another.

Because the adjusted estimates, when the diurnal amplitude is based on the current year only, have more or less the same properties as those based on day catches only, it might be a proper strategy to restrict surveys to day if all species and length groups of interest exhibit strong year-to-year differences in diurnal variation. This is not the case for the Barents Sea surveys, and a 24-h survey strategy is adequate because more information is then obtained for medium- and large-sized fish.

Even though standard bottom trawl survey results have worked quite well for stock assessment in the past (Korsbrekke et al. 2001), new demands may in future call for more correct estimation of the absolute level. Particularly, correct density relations among size groups and species will be important in more advanced ecosystem approaches that demand correct data on trophic relationships. As diurnal behaviour dynamics are important for intra- and inter-specific relationships (e.g., Neilson and Perry (1990) and references therein), it will be important to establish models that can adjust for the associated bias. This may potentially improve our understanding of the ecosystem function and also enhance the efficiency of more advanced assessment models. Such adjustments could also improve the acoustic assessment because information from trawl catches normally is applied to convert acoustic back-scattering cross section to fish

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densities by species and size. Finally, our results should be taken into account in the design of future survey strategies. This will be particularly important when combining density information from trawl catches with simultaneous density records from the acoustic method.

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Appendix A

Details of the models in Models section

The apparent local time t in eq. 1 is calculated as $t = t_{UTC} + \text{longitude}/15 + \Delta$, where $\Delta = [-0.4083\sin(0.0172(\text{day} - 80)) - 1.7958\cos(0.0172(\text{day} - 80)) + 2.4875\sin(0.0344(\text{day} - 80))]/15$, with $1 \le \text{day} \le 365$ being the day of the year, is an approximation to the equation of time (Smart 1977, p. 150).

If the parameters α and β are kept fixed in eq. 3, then eq. 1 may be replaced by the linear model

$$\text{(A1)} \quad y_i = \mu_{d(i)} + D f_L^*(t_i) + \varepsilon_i$$

where

$$f_L^*(t_i) = f_L(t_i; D = 1, \alpha = \alpha_0, \beta = \beta_0)$$

with α_0 and β_0 being appropriately chosen constants. To get separate estimates of D for each year, we may write

$$\text{(A2)} \quad y_i = \mu_{d(i)} + D_{a(i)} f_L^*(t_i) + \varepsilon_i$$

where $a(i)$ is the year that haul i is taken. If the sinusoidal function is preferred, $f_L^*(t_i)$ in eqs. A1 and A2 can be replaced by $f_S^*(t_i) = f_S(t_i; D = 1)$.

Also, the nonlinear model may be refined to yield separate estimates of D for each year by simply replacing D in eq. 3 by $D_{a(i)}$. Then α and β can be estimated using data for all years, whereas $D_{a(i)}$ is estimated using data from year $a(i)$ only. In the section Yearly estimates of diurnal variation, we have used the nonlinear version with $\alpha = 2$ fixed, that is,

$$\text{(A3)} \quad y_i = \mu_{d(i)} + f_L(t_i; D = D_{a(i)}, \alpha = 2, \beta) + \varepsilon_i,$$

whereas for the bootstrap experiments in the section Adjusting for diurnal effects, we used eq. A2.

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Stylized example illustrating the effect of uncertainty on bias correction

Let μ_N and μ_D be the nighttime and daytime levels of a pure threshold (night–day levels only) model, and let nighttime measurements $z_{i,N}$ and daytime measurements $z_{i,D}$ have expectations $E(z_{i,N}) = \mu_N$ and $E(z_{i,D}) = \mu_D$, respectively, with a common variance $\text{var}(z_{i,N}) = \text{var}(z_{i,D}) = \sigma^2$. If there are n_N nighttime measurements and n_D daytime measurements, then $\mu_N - \mu_D$ is estimated by

$$\bar{z}_N - \bar{z}_D = n_N^{-1} \sum_{i=1}^{n_N} z_{i,N} - n_D^{-1} \sum_{i=1}^{n_D} z_{i,D}$$

and the adjusted measurements are given by

$$z_{i,\text{adj}} = \begin{cases} z_{i,D} & \text{for daytime measurements} \\ z_{i,N} - (\bar{z}_N - \bar{z}_D) & \text{for nighttime measurements} \end{cases}$$

It is then trivial to check that $\bar{z}_{\text{adj}} = (n_N + n_D)^{-1} \sum_i z_{i,\text{adj}} = \bar{z}_D$ so that the adjusted estimate and the daytime estimate are identical and therefore have the same error, irrespective of the values of n_N and n_D.

Testing for model fitness

Tests of the hypotheses $f = C$ (constant), $f = f_S$, and $f = f_L$ were executed by computing functionals of the data

$$\sum_i \{\hat{f}(t_i) - \hat{C}\}^2; \quad \sum_i \{\hat{f}(t_i) - \hat{f}_S(t_i)\}^2; \quad \sum_i \{\hat{f}(t_i) - \hat{f}_L(t_i)\}^2$$

measuring the distances between the nonparametric estimate $\hat{f}$ and the parametric estimates $\hat{C}$, $\hat{f}_S$, and $\hat{f}_L$ under the null hypotheses $f = C$, $f = f_S$, and $f = f_L$. The hypotheses are rejected for large values of the functionals, the null distribution of these functionals being established by bootstrapping. For more details, we refer to Hjellvik and Tjøstheim (1995) and Hjellvik et al. (2001). In general α, β, and D were all estimated under H_0: $f = f_L$, but in one case (deep-sea redfish), $\alpha = 2$ were kept fixed because of convergence problems with the nls algorithm.

It should be noted that our test of $f = C$, i.e., whether there are diurnal variations or not, does not suffer from the bias relative to a randomized procedure, observed for the test in Casey and Myers (1998). This is because we use the bootstrap principle so that the randomization is inherent in the test itself.

Testing for annual differences in diurnal variation

We have used Simes modified Bonferroni procedure (Simes 1986) to test for annual differences. This is an improved version of the Bonferroni correction for conducting multiple tests of significance. If $H = \{H_1,\ldots,H_n\}$ is a set of null hypotheses with corresponding test statistics $T_1,\ldots,T_n$, p values $P_1,\ldots,P_n$, and H_0 is the hypothesis that H_i, $i = 1,\ldots,n$ are true, then the suggested procedure rejects H_0 at level α if and only if

(A4) there exists some value of $j (1 \le j \le n)$ such that $P_{(j)} \le j\alpha/n$

where $P_{(1)},\ldots,P_{(n)}$ are the ordered values of $P_1,\ldots,P_n$.

This procedure can be applied to our situation. Let $\{D_1,\ldots,D_m\}$ denote the true values of D for the m years where estimates of D are available for the actual length group and species. The null hypothesis H_0: $D_1 = \ldots = D_m$ can then be restated as H_0: $\delta_{ij} \doteq D_i - D_j = 0$, $i = 1,\ldots, m-1$, $j = i+1,\ldots,m$. The global null hypothesis H_0 is then true if and only if all of the $n = m(m-1)/2$ null hypotheses $\{H_{ij}$: $\delta_{ij} = 0\}$ are true. Under H_{ij}, we have that $E(\delta_{ij}) = 0$ and $\text{var}(\delta_{ij}) = \sigma_i^2 + \sigma_j^2$, where $\sigma_i^2 = \text{var}(D_i)$, $i = 1,\ldots,m$. We assume that the test statistic $T_{ij} = (\hat{D}_i - \hat{D}_j)/(s_i^2 + s_j^2)^{1/2}$, where s_i is the standard error for $\hat{D}_i$ yielded by the function lm in S-PLUS, follows a standard normal distribution. The p value for T_{ij} is then calculated as $P_{ij} = 2\min(S_{ij}, 1 - S_{ij})$, where $S_{ij} = \Phi(T_{ij})$ and Φ is the standard normal distribution function. The global p value is the lowest value of α for which eq. A4 is fulfilled ($P_{(1)},\ldots,P_{(n)}$ being the ordered values of $\{P_{ij}\}$).

Simes modified Bonferroni test appears particularly advantageous compared with the classical Bonferroni procedure when several highly correlated test statistics are involved (Simes 1986). In our situation, many of the test statistics are positively correlated since $\text{cov}(D_i - D_j, D_i - D_k) = \text{var}(D_i)$ for $i \ne j \ne k$. Still, the actual level of the test is acceptable. Using a nominal level of 5% and 10 000 replicates, we simulated D_is from the $N(0, s_i^2)$ distribution for all species or length groups with $m > 3$ and got empirical levels ranging from 0.0344 to 0.0450, with an average of 0.0391.

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Hydroelectric development and translocation of *Galaxias brevipinnis*: a cloud at the end of the tunnel?

Jonathan M. Waters, Michael Shirley, and Gerard P. Closs

Abstract: Two major drainages of southeastern Australia, the Snowy River and the Murray River, were artificially linked by a major hydroelectric project during the early 20th century. This development diverts Snowy River flow into tributaries of the Murray River via a series of extensive tunnels. In 1990, fish surveys of the upper Murray River system recorded *Galaxias brevipinnis*, an aggressive migratory species previously unrecorded from the drainage. We used genetic analysis to discriminate between alternative hypotheses for Murray River *G. brevipinnis*: (*i*) anthropogenic translocation via the Snowy River diversion or (*ii*) a previously undiscovered natural population. Landlocked *G. brevipinnis* from the Murray River (43 fish, eight control region haplotypes) and Snowy River (39 fish, 11 haplotypes) exhibit similar levels of mtDNA diversity, share six haplotypes, and are not significantly differentiated for microsatellite loci ($p = 0.0884$). Coastal samples exhibit higher haplotypic diversity (40 fish, 20 haplotypes) but share only three haplotypes with Murray River and are significantly differentiated from Murray River samples for microsatellite loci ($p = 0.0008$). Our data are consistent with the translocation hypothesis but are generally inconsistent with a natural origin for Murray River *G. brevipinnis*. The suggested human-mediated translocation represents a risk to native fauna.

Résumé : Deux importants bassins hydrographiques du sud-est de l'Australie, ceux des rivières Snowy et Murray, ont été connectés artificiellement lors de l'établissement d'un projet hydroélectrique de grande envergure au début du 20e siècle. Le débit de la rivière Snowy a été détourné vers les tributaires de la rivière Murray à l'aide d'un vaste réseau de tunnels. En 1990, l'étude des poissons dans l'amont du réseau hydrographique de la rivière Murray a observé pour la première fois la présence de *Galaxias brevipinnis*, une espèce invasive. L'analyse génétique nous a permis d'évaluer deux hypothèses pour expliquer la présence de *G. brevipinnis* dans la Murray, soit (*i*) une invasion du à l'activité humaine du au détournement de la Snowy, soit (*ii*) l'existence d'une population naturelle pré-existante jamais observée. *Galaxias brevipinnis* de la Murray (43 poissons, 8 haplotypes sur la région de contrôle) et la Snowy (39 poissons, 11 haplotypes) montrent des niveaux de diversité similaires pour de l'ADNmt, ils partagent six haplotypes et ils ne se distinguent pas de façon significative sur la base des locus microsatellites ($p = 0{,}0884$). Les poissons provenant de la côte ont une plus grande diversité haplotypique (40 poissons, 20 haplotypes), mais ne possèdent que trois haplotypes communs avec les poissons de la Murray. Ils se différencient significativement des poissons de rivière sur la base des locus microsatellites ($p = 0{,}0008$). Nos données appuient généralement l'hypothèse d'une translocation mais s'accordent assez mal avec celle de l'origine naturelle des *G. brevipinnis* de la Murray. Cette translocation causée par activité humaine, suggérée par notre étude, représente un risque pour la faune indigène.

[Traduit par la Rédaction]

Introduction

Climbing galaxias (*Galaxias brevipinnis*) is a freshwater fish found in southeastern Australia, Tasmania, New Zealand, and associated islands (McDowall 1990). This species is an aggressive upstream migrant that is able to penetrate long distances inland (McDowall and Fulton 1996). The migratory behaviour of *G. brevipinnis* is promoted by a remarkable climbing ability: it is known to climb vertical waterfalls that are tens of metres high (McDowall 1990). Most populations of *G. brevipinnis* are diadromous: they have a migratory marine juvenile phase that is thought to facilitate dispersal. However, there are numerous lake populations that complete their entire life cycle in freshwater. For instance, a large number of isolated postglacial lakes have been successfully colonised by this species (McDowall 1990). Migratory taxa are often characterised by extensive geographic ranges and relatively little population genetic differentiation: a form of evolutionary stasis (e.g., Allibone and Wallis 1993; Waters et al. 2000*a*). In contrast, fresh-

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J.M. Waters[1] **and G.P. Closs.** Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand.
M. Shirley. Sinclair Knight Merz, P.O. Box 2500, Malvern, Victoria, 3144 Australia.

[1]Corresponding author (e-mail: jonathan.waters@toroa.otago.ac.nz).

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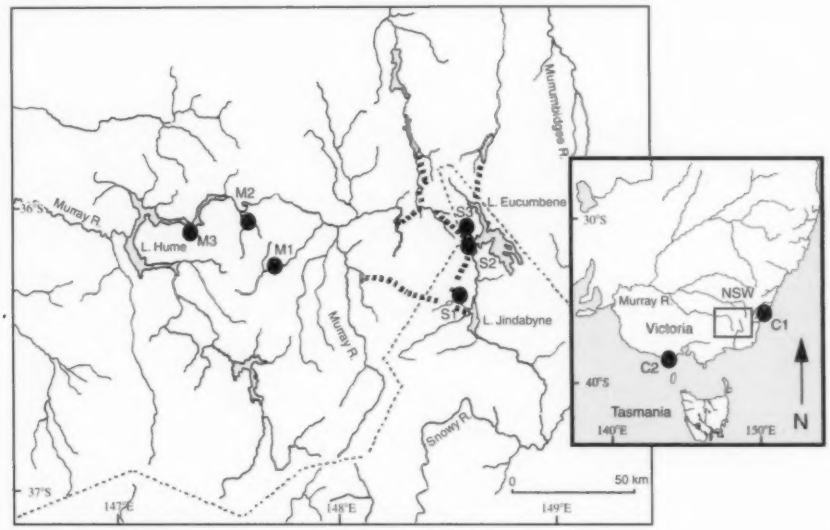
Fig. 1. Map of southeastern Australia showing the eight sampling locations for *G. brevipinnis*. The inland–coastal drainage divide is indicated by a thin broken line. Thick broken lines indicate water tunnels that artificially connect the drainages of the Snowy, Murray, and Murrumbidgee rivers.



water-limited populations have the potential for diversification (Waters and Wallis 2001*a*). The genetic relationships and conservation significance of landlocked *G. brevipinnis* remain poorly understood.

Galaxias brevipinnis occurs in most coastal rivers of southeastern Australia but is almost entirely absent from Australia's major inland drainage, the Murray River (Fig. 1). Indeed, the species was unknown from the inland system until a recent survey of the upper Murray River (Morison and Anderson 1991) recorded it from a number of tributaries draining in and around the Lake Hume reservoir (Fig. 1). Murray River populations of *G. brevipinnis* are currently sympatric with the congener *G. olidus*, a stream-resident species that is native to the system (McDowall and Frankenberg 1981). Adults of the two species are morphologically similar but can be distinguished on the basis of pectoral ray counts and head morphology (McDowall and Fulton 1996). Pre-1990 surveys of the upper Murray River were extensive but yielded no *G. brevipinnis* (McDowall and Frankenberg 1981). Morison and Anderson (1991) reexamined previous Murray River collections and confirmed the absence of *G. brevipinnis*. They concluded that Murray River *G. brevipinnis* "are most probably recently introduced rather than being undiscovered natural populations".

Morison and Anderson (1991) and McDowall and Fulton (1996) both suggested that the Snowy Mountains hydroelectric scheme was a possible means of recent translocation. This development annually diverts 500 to 1000 GL of Snowy River flow into tributaries of the Murray River. Specifically, headwaters of the Snowy River are impounded in man-made lakes Eucumbene and Jindabyne (Fig. 1) and are diverted across the Great Dividing Range into the Murray River through tunnels that are up to 23.5 km in length (Fig. 1). Morison and Anderson (1991) considered that "fish would have little difficulty moving through these tunnels: screens on tunnel entrances are large relative to fish size, and the tunnel gradients are generally small".

Life-history data indicate that the Murray River populations are landlocked (Morison and Anderson 1991). Indeed, an ongoing (annual) marine phase is almost inconceivable for these populations given the 2225-km migration required. Additionally, the Murray River is now disrupted by two major dams and 13 weirs, blocking the path of any potential upstream migrants. Therefore, recent upriver migration is a highly unlikely explanation for the presence of *G. brevipinnis* high in the Murray River.

Although circumstantial evidence for recent translocation of Snowy River *G. brevipinnis* into the Murray River is compelling, it remains possible that the newly identified populations of *G. brevipinnis* are native to the drainage. Colonisation may have occurred through upriver migration (before dam construction) or river capture. If this is the case, it is possible that they comprise an Evolutionarily Significant Unit (ESU; Moritz 1994). Indeed, the Murray River is known to house substantial biodiversity in other freshwater fish groups (e.g., Ovenden et al. 1988). Furthermore, genetic studies have revealed the presence of substantial cryptic diversity in nonmigratory derivatives of New Zealand *G. brevipinnis* (Allibone and Wallis 1993). As a result, a number of new species have recently been described (e.g., McDowall and Wallis 1996).

The current study aims to determine the genetic status of Murray River *G. brevipinnis* and thereby discriminate between conflicting hypotheses explaining their origin. Under a translocation scenario, we would expect Snowy River and Murray River samples to exhibit similar haplotypic and genotypic compositions. Alternatively, under an independent origin scenario, we expect substantial genetic divergence between Snowy River and Murray River samples. If human-mediated translocation is supported, our study may have implications for hydroelectric developments in general. Al-

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Table 1. Geographic details, life-history status, and timing of *Galaxias* samples.

Stream	Drainage	Life history	Code	*n*	Date	Latitude	Longitude
Macquarie Rivulet	(Coastal)	Diadromous	C1	30	May 2000	35°25′S	150°42′E
Jamison Creek	(Coastal)	Diadromous	C2	10	May 2000	38°36′S	143°55′E
Diggers Creek	Snowy River	Landlocked	S1	16	June 2000	36°20′S	148°29′E
Portal Inlet	Snowy River	Landlocked	S2	6	Feb. 2001	36°05′S	148°35′E
Big Tolbar Creek	Snowy River	Landlocked	S3	20	Feb. 2001	36°03′S	148°34′E
Cudgewa Creek	Murray River	Landlocked	M1	28	June 2000	36°08′S	147°38′E
Guys Forest Creek	Murray River	Landlocked	M2	27	June 2000	36°07′S	147°30′E
Cottontree Creek	Murray River	Landlocked	M3	30	June 2000	36°07′S	147°20′E

Note: Location codes are used in Fig. 1.

ternatively, if Murray River *G. brevipinnis* are shown to be native to this drainage, we aim to identify significant units of biodiversity for conservation.

Materials and methods

One hundred and sixty-seven *Galaxias* specimens were caught from eight geographic locations (Fig. 1; Table 1) with the aid of electric fishing apparatus. Two of the samples were from coastal (diadromous) populations: Macquarie Rivulet (C1) and Jamison Creek (C2). Three samples were collected from streams draining into Snowy River impoundments: Lake Jindabyne (Diggers Creek, S1) and Lake Eucumbene (Big Tolbar Creek, S2; Portal Inlet, S3). Finally, three samples were collected from tributaries of Murray River (Cudgewa Creek, M1; Guys Forest Creek, M2; Cottontree Creek, M3). Fish were anaesthetised, and nonlethal tissue samples were removed (caudal fin clips; 3–4 mm in diameter) and stored in absolute ethanol at 4°C. The taxonomic status of some juvenile specimens from Murray River sites (M1–M3) was difficult to confirm on the basis of morphology; these samples were classified according to mtDNA type (see below).

Total DNA was extracted from fin tissue of all 167 specimens using a CTAB (cetyltrimethylammonium bromide) – proteinase K digestion buffer and was subsequently used for both microsatellite and mtDNA analyses. Initially, the species identity of *Galaxias* samples was determined by RFLP (restriction fragment length polymorphism) analysis of mtDNA. This entailed amplification of the complete mitochondrial cytochrome *b* gene (1160 base pairs (bp); see Waters and Wallis 2001*a*) followed by digestion with four-base cutters *Hae* III and *Hinf* I. Restriction fragment patterns were resolved using agarose electrophoresis. Representative cytochrome *b* digestion patterns were sequenced and compared with published sequences (Waters et al. 2000*b*) to confirm identity. Three specimens from Big Tolbar Creek failed to amplify and were excluded from subsequent analyses. The 123 specimens confirmed as *G. brevipinnis* were included in subsequent population genetic analyses, whereas the 41 samples identified as *G. olidus* were excluded from *G. brevipinnis* analyses. A 740-bp fragment of the mitochondrial control region was sequenced for 93 of the 123 *G. brevipinnis* specimens. The remaining 30 specimens of *G. brevipinnis* were not sequenced because of financial constraints. Polymerase chain reaction (PCR) amplification and sequencing of the control region were carried out using primers P4 and S-Phe, as specified in Waters and Wallis 2001*b*. Sequences were aligned by eye.

One hundred and sixty-four *Galaxias* samples were genotyped for three microsatellite loci: Gvu4, Gvu5, and Gvu7 (primers and PCR conditions presented in Waters et al. (1999)). These loci contain tetranucleotide $(GAAA)_9$, trinucleotide $(GTT)_7(GCT)_3$, and dinucleotide $(GA)_{24}$ repeats, respectively. PCR amplifications were carried out in 15 μL volumes using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Mass.), and contained 0.5 U of Taq DNA polymerase (Roche Mannheim). Amplified products were run on 8–10% nondenaturing polyacrylamide gels at 100 V for 16–20 h using a Protean electrophoresis system (BioRad Laboratories, Hercules, Calif.). Gels were stained with SYBR Green 1 (Molecular Probes Inc., Eugene, Oregon) and photographed under ultraviolet light. Allele sizes were determined by electrophoretic comparison with a 20-bp molecular ruler (BioRad).

Statistical analyses

Microsatellite allele frequencies and heterozygosity were calculated using GENEPOP 3.1b (Raymond and Rousset 1997). Tests for deviation from Hardy–Weinberg equilibrium were performed with the Markov chain method (Guo and Thompson 1992) implemented in GENEPOP (Raymond and Rousset 1995). Tests for allelic differentiation among samples were performed with GENEPOP using Fisher's exact test (Raymond and Rousset 1995) with the following parameters: dememorization 3000, batches 150, iterations 3000. Nei's (1978) genetic distance was calculated for all sample pairs based on microsatellite data using Genetix v4.01 (Belkhir et al. 1999).

Distinct control region haplotypes were identified using phylogenetic software (PAUP* version 4.0b4; Swofford 1998), and haplotype frequencies were calculated manually. Tests for haplotypic differentiation among samples were performed with GENEPOP. A minimum spanning tree of haplotype relationships was constructed with Arlequin version 2.000 (Schneider et al. 2000) using absolute distances. Pairwise F_{ST} values based on haplotypic data, incorporating Kimura (1980) two-parameter distances, were calculated with Arlequin. Multidimensional scaling (MDS) of interpopulational F_{ST} values and Nei's distances was performed using Primer 5 version 5.2.3 (Clarke and Warwick 2001).

Results

Mitochondrial DNA

Control region sequences (740 bp) of *G. brevipinnis* revealed no length variation, making sequence alignment straightforward. A total of 93 specimens was sequenced

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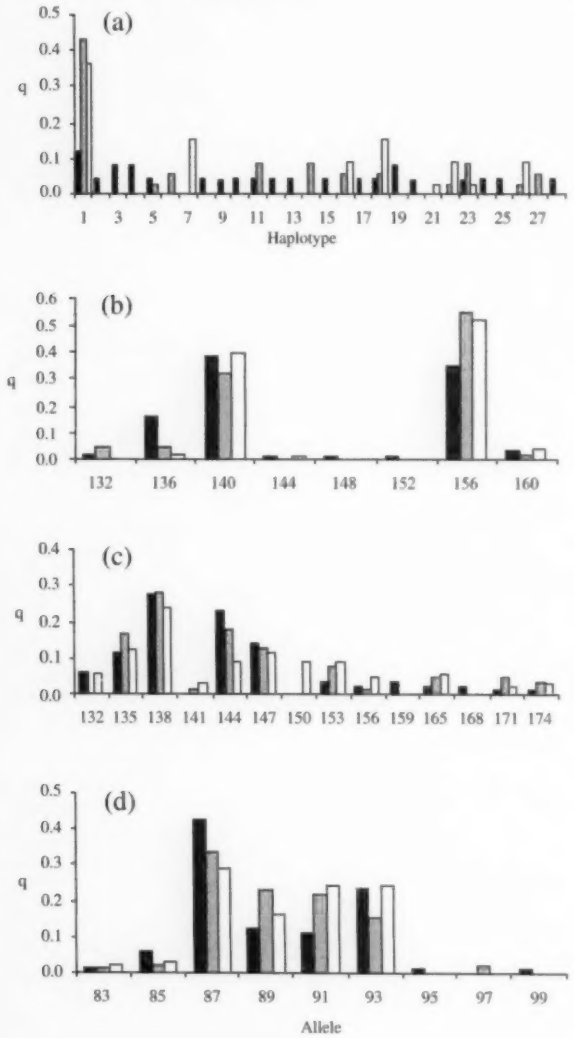
Fig. 2. Histograms showing (*a*) haplotype frequencies for mtDNA control region and (*b–d*) allele frequencies for three microsatellite loci: (*b*) Gvu4, (*c*) Gvu5, and (*d*) Gvu7. Samples from Coastal, Snowy, and Murray rivers are represented by solid, shaded, and open bars, respectively.



(GenBank accession AF413216–307), yielding 28 distinct haplotypes (Fig. 2). Variation was detected at 36 nucleotide positions, of which 30 were located in the first 300 bp (5′ region) of the control region. Most nucleotide substitutions were transitions, with transversions detected at only three of the 36 variable sites.

Haplotypic diversity within samples ranged from 0.93 (C1) to 0.47 (M3). Diversity was extremely high in coastal samples (C1–C2; 25 sequences; 20 haplotypes; Table 2), lower in artificially landlocked Snowy River samples (S1–S3; 35 sequences; 11 haplotypes), and lowest of all in the Murray River samples (M1–M3; 33 sequences; eight haplotypes; Table 2). Absolute differences between haplotypes ranged from 1 to 15 substitutions (Fig. 3).

Fig. 3. Minimum spanning tree showing possible relationships between the 28 control region haplotypes. Shaded circles represent haplotypes recorded in coastal samples, whereas open circles represent haplotypes detected only in the Murray River (M) and Snowy River (S) drainages. "Missing" haplotypes are represented by the symbol ○. Three coastal haplotypes were also found in the catchments of both the Snowy and Murray rivers (haplotypes 1, 18, and 23). The most common haplotype (1) is enlarged; other haplotypes with high overall frequencies are moderately enlarged.

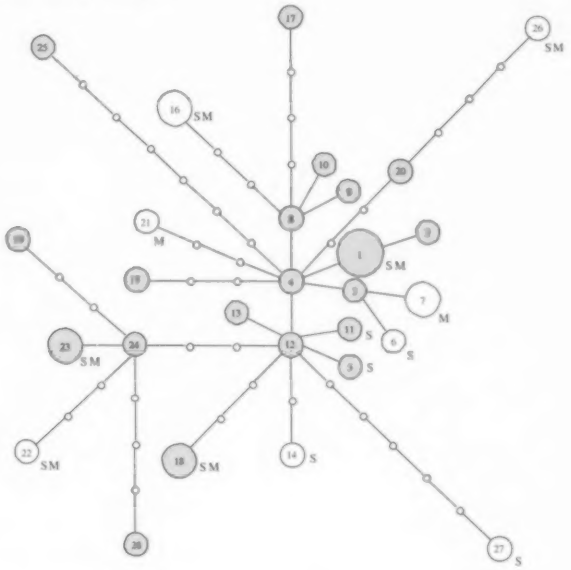
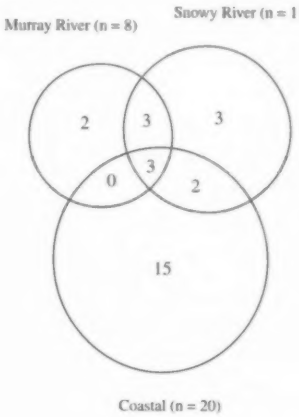


Fig. 4. Venn diagram indicating geographic distribution of the 28 control region haplotypes. Six of 11 Snowy River haplotypes were recorded in Murray River samples, whereas just three of 20 coastal haplotypes were detected in the Murray River samples.



Nearly half (13) of the 28 haplotypes were recorded only once. Thirteen of the remaining 15 haplotypes were recorded

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Table 2. Number of control region haplotypes and haplotypic diversity (H_e) for each *Galaxias brevipinnis* sample.

Source	Sample	Code	Sequences	Haplotypes	H_e
Coastal (diadromous)	Macquarie Rivulet	C1	16	15	0.93
	Jamison Creek	C2	9	8	0.86
	Pooled		25	20	0.90
Snowy River (landlocked)	Diggers Creek	S1	14	8	0.77
	Portal Inlet	S2	6	4	0.67
	Big Tolbar Creek	S3	15	4	0.74
	Pooled		35	11	0.72
Murray River (translocated?)	Cudgewa Creek	M1	11	7	0.74
	Guys Forest Creek	M2	13	7	0.84
	Cottontree Creek	M3	9	4	0.47
	Pooled		33	8	0.68

Note: Values for pooled populations are also given.

Fig. 5. Multidimensional scaling of pairwise F_{ST} values for the eight samples based on Kimura (1980) two-parameter distances among mtDNA control region sequences. Circles link sample categories.

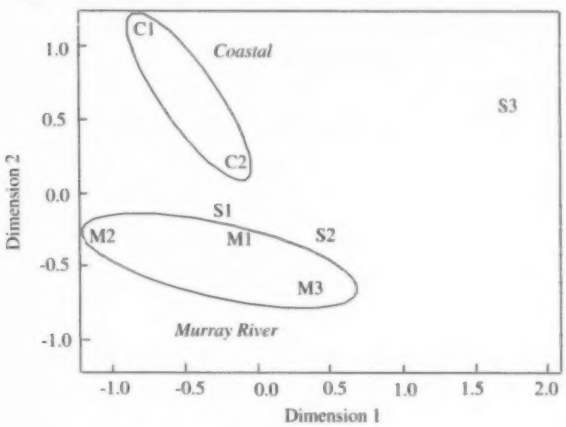
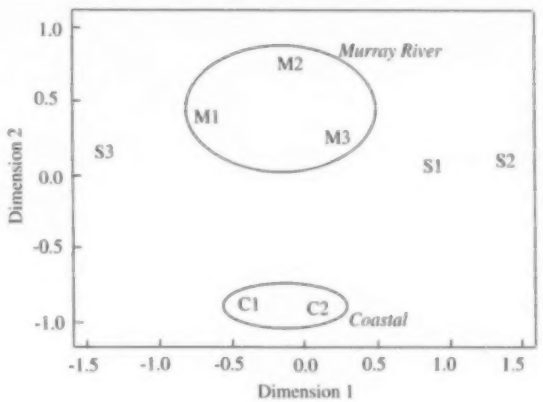


Fig. 6. Multidimensional scaling of Nei's (1978) distances among the eight samples based on allele frequencies at the three microsatellite loci. Circles link sample categories.



from multiple sample sites. One particularly common haplotype (haplotype 1; Figs. 2, 3) was detected in all eight sample locations. This haplotype occurred at low frequency in coastal samples (0.12) but at high frequency in Snowy River (0.46) and Murray River samples (0.36). Six haplotypes were shared by Murray River and Snowy River samples (Fig. 4), whereas only three were shared by Murray River and coastal samples (Fig. 4). The six haplotypes common to the Snowy River and Murray River were quite divergent from one another. As these shared haplotypes were drawn from a diverse gene pool (Fig. 3), the possibility that the associated populations were independently derived seems very weak.

No significant mtDNA differentiation was observed within coastal (C1–C2), Snowy River (S1–S3), or Murray River sample groups (M1–M3). However, when samples were pooled into coastal, Snowy River, or Murray River groups, significant mtDNA differentiation was observed for all comparisons: Murray River versus Snowy River ($p = 0.0212$); Murray River versus coastal samples ($p < 0.0001$); Snowy River versus coastal samples ($p = 0.0005$). Multidimensional scaling of F_{ST} values among samples (Fig. 5) confirms that Murray River samples are closely allied to Snowy River samples S1 and S2.

Microsatellite DNA

Microsatellite DNA analysis clearly discriminated *G. brevipinnis* and *G. olidus*, yielding results completely congruent with mtDNA-based allocation (data not shown). In particular, the dinucelotide repeat Gvu7 was highly discriminatory, with overlapping allele size ranges for the two taxa (83–99 bp for *G. brevipinnis*, Fig. 2; >120 bp for *G. olidus*). The genotypic data yielded no evidence of any F1 hybrids.

Galaxias brevipinnis ($n = 123$) exhibited considerable microsatellite diversity: Gvu4, Gvu5, and Gvu7 yielded 8, 14, and 9 alleles, respectively (Fig. 2). Allelic diversity was highest in diadromous samples ($n = 40$, 28 alleles, mean $H_e = 0.76$; Table 3). However, the contrast between diadromous and landlocked samples was less marked than that observed for mtDNA: Snowy River and Murray River samples yielded 22 and 23 alleles, respectively (Table 3).

Hardy–Weinberg exact tests (24 sample-locus combinations) yielded just a single case of significant deviation from equilibrium (Gvu4, C2; $p = 0.0028$). There was no signifi-

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Table 3. Number of alleles and expected heterozygosity (H_e) for each microsatellite locus in each *Galaxias brevipinnis* sample.

Source	Sample (n)	Code	Locus	Gvu4	Gvu5	Gvu7	Total	Mean
Coastal (diadromous)	Macquarie Rivulet (30)	C1	Alleles	7	12	8	27	
			H_e	0.70	0.85	0.75		0.77
	Jamison Creek (10)	C2	Alleles	5	6	4	15	
			H_e	0.74	0.81	0.69		0.75
	Pooled (40)		Alleles	8	12	8	28	
			H_e	0.72	0.81	0.72		0.76
Snowy River (landlocked)	Diggers Creek (16)	S1	Alleles	3	8	6	17	
			H_e	0.50	0.81	0.78		0.70
	Portal Inlet (6)	S2	Alleles	3	8	5	16	
			H_e	0.44	0.92	0.80		0.72
	Big Tolbar Creek (17)	S3	Alleles	5	7	5	17	
			H_e	0.64	0.70	0.77		0.70
	Pooled (39)		Alleles	5	10	7	22	
			H_e	0.53	0.81	0.78		0.71
Murray River (translocated?)	Cudgewa Creek (13)	M1	Alleles	3	8	6	17	
			H_e	0.56	0.84	0.82		0.74
	Guys Forest Creek (20)	M2	Alleles	4	11	5	20	
			H_e	0.56	0.91	0.77		0.75
	Cottontree Creek (11)	M3	Alleles	4	7	5	16	
			H_e	0.64	0.86	0.75		0.75
	Pooled (44)		Alleles	5	12	6	23	
			H_e	0.59	0.87	0.78		0.75

Note: Values for pooled populations and across all three loci are also given.

Table 4. Probability of allelic differentiation among samples, calculated for each microsatellite locus, and across all loci, using Fisher's exact test (Raymond and Rousset 1995).

Population comparison	Gvu4	Gvu5	Gvu7	Overall
Murray River versus Snowy River	0.1712	0.0561	0.5230	0.0884
Murray River versus coastal	0.0044**	0.0130*	0.1598	0.0008***
Snowy River versus coastal	0.0439*	0.1502	0.0807	0.0203*

Note: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

cant deviation from Hardy–Weinberg expectations across all loci and across populations ($p = 0.1307$).

Across all three microsatellite loci, no significant allelic differentiation was observed within coastal (C1–C2) or Murray River sample groups (M1–M3). However, significant differentiation was observed for two Snowy River sample comparisons (S3 versus S2, $p = 0.0028$; S3 versus S1, $p < 0.0001$; see Fig. 6). Multidimensional scaling of Nei's (1978) distances among samples illustrates that Murray River samples are closely allied to Snowy River samples (Fig. 6). In fact, the Snowy River sample S3 is genotypically more distinct from other Snowy River samples (S1, S2) than it is from the Murray River samples (M1–M3). When samples were pooled into coastal, Snowy River, or Murray River groups, differentiation for Murray River versus Snowy River was significant ($p = 0.0884$; Table 4), whereas significant differentiation was observed for Murray River versus coastal samples ($p = 0.0008$) and for Snowy River versus coastal samples ($p = 0.0203$; Table 4).

Discussion

Our genetic data are consistent with the Snowy River – Murray River translocation hypothesis but largely inconsistent with the alternative scenario involving an older independent origin for Murray River *G. brevipinnis*. Specifically, the haplotypic and genotypic similarity of landlocked Snowy River and Murray River samples is greater than would be expected by chance if these populations were independently derived from coastal populations. The finding that six of eight Murray River haplotypes are present in Snowy River impoundments suggests a recent connection between the two populations and argues against the hypothesis that the former population represents a previously undiscovered ESU (Moritz 1994).

Although genetic data are consistent with the a priori hypothesis of human-mediated translocation, conventional mtDNA divergence rates (2% per million years; Brown et al. 1982) imply that shared haplotypes cannot discriminate between contemporary (20th century) and prehistoric (e.g., early Holocene) biogeographic events. Thus, we cannot completely reject natural phenomena (e.g., recent river capture; bird-mediated dispersal; storm-mediated dispersal) as possible alternative mechanisms of transalpine translocation. Nevertheless, we are wary of erecting post hoc biogeographic hypotheses that have no independent support. For instance, Bishop (1995) suggested that river capture is a rare geological process that should not be invoked without

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geological evidence. As the catchments of the Murray and Snowy rivers are artificially connected, we contend that it is unscientific to invoke additional biogeographic processes (e.g., river capture) for which there is no evidence.

Although there is significant mtDNA differentiation between the Murray River and Snowy River samples (p = 0.0196), this is not unexpected for a translocation event involving relatively few founding individuals, especially given the small effective population size of mtDNA (Ovenden and White 1990). The finding that Snowy River and Murray River samples are not significantly differentiated for microsatellite loci (p = 0.0884), whereas both are distinct from coastal samples, further supports the translocation hypothesis. Interestingly, both mtDNA and microsatellite data indicate that Snowy River samples S1 and S2 are more distinct from the third Snowy River sample (S3) than they are from Murray River samples (M1–M3). This result might be consistent with a source (Snowy River) and sink (Murray River) scenario involving multiple artificial connections between the two drainages.

Mitochondrial DNA data suggest that the Murray River population was founded by at least eight female *G. brevipinnis* (eight haplotypes). However, the significant mtDNA differentiation between Snowy River and Murray River samples implies that interchange between the two drainages is limited. We suggest that dispersal through man-made water tunnels (some over 20 km in length) is sporadic but perhaps ongoing. Although dispersal through these tunnels may seem improbable, it is consistent with the migratory capacity of this species (McDowall 1990). Despite their apparently recent origin, the geographic range of Murray River *G. brevipinnis* is already considerable, extending some 150 km west of the Snowy–Murray drainage divide (Morison and Anderson 1991). More recent records of *G. brevipinnis* from the Ovens and Goulburn catchments of the Murray drainage (G.P. Closs, unpublished data; T. Raadik, Arthur Rylah Institute, 123 Brown Street, Heidelberg VIC 3084, Australia, unpublished data) further emphasize their migratory ability.

The biological implications of hydroelectric developments have often been largely ignored in the face of more immediate and transparent economic concerns. When man-made watercourses violate long-established natural drainage boundaries, there is a high risk of faunal translocation. If introduced species are closely related to native taxa, artificial translocation has the potential to reduce biodiversity through hybridization, a process that may be highly cryptic (Echelle and Echelle 1997). For instance, interdrainage diversion of water as a result of 19th century mining practices in New Zealand resulted in secondary contact of related *Galaxias* taxa, with associated hybridization and introgression (Esa et al. 2001). Artificial drainage connections have led to similar concerns in North America (Minckley 1978). Associated negative impacts may be in the form of genetic assimilation (Ellstrand 1992), the breakup of co-adapted gene complexes, and reduction in adaptation to local conditions (Templeton 1986).

The translocation of more divergent taxa may lead to local elimination or complete extinction of native fauna. For example, the construction of the Lake Pedder impoundment in Tasmania during the 1970s and the establishment of an artificial connection with the adjacent Gordon River system were accompanied by invasions of brown trout and *G. brevipinnis*. It is thought that the predatory and competitive behaviours of these translocated taxa were largely responsible for the subsequent decline in the abundance of the endemic *Galaxias pedderensis* (McDowall and Fulton 1996; Crook and Sanger 1997), which is now considered the most endangered freshwater fish in Australia (Hamr 1995).

The risks associated with translocation of exotic fishes are well known. Introduced Nile perch (*Tilapia*) have disastrously impacted the unique and diverse cichlid faunas of central African lakes (Avise 1990). Introduced *Tilapia* and *Gambusia* have also adversely affected native cyprinid and poeciliid fishes of the western United States (Meffe 1985; Vrijenhoek 1996). Deliberate introductions of northern hemisphere fish species such as brown trout (*Salmo trutta*) have greatly reduced the distribution and abundance of endemic fish in New Zealand (Townsend and Crowl 1991) and Australia (McDowall and Fulton 1996). Many nonmigratory galaxiid species are now restricted to streams above waterfalls that are impassable to trout (e.g., Australia: *G. fontanus*, *G. olidus*; New Zealand: *G. depressiceps*, *G. pullus*, *G. eldoni*).

The construction of impoundments may profoundly alter aquatic communities. Taxa that are able to exploit lacustrine conditions may outcompete taxa that are less versatile. African *Tilapia* flourish in impoundment conditions and often spread to surrounding stream habitats (Vrijenhoek 1996). Diadromous *G. brevipinnis* exhibit similar plasticity in life history: they readily switch to a nonmigratory life history in impoundments, with oceanic larval development replaced by a lacustrine juvenile phase (Allibone 1999). The construction of the Lake Mahinerangi impoundment in the Waipori catchment (New Zealand) promoted the local proliferation of *G. brevipinnis* (McDowall and Allibone 1994) and substantially reduced the range and abundance of stream-resident endemics *G. eldoni* and *G. pullus* (McDowall 1997).

In conclusion, the probable anthropogenic translocation of *G. brevipinnis* into the Murray River poses a threat to the native fauna of this drainage. There is a risk of hybridization between translocated *G. brevipinnis* and native *G. olidus*. Although our preliminary data provide no evidence of any F1 hybrids for these taxa, future studies should examine this issue in detail. In addition, future studies should monitor both the spread of *G. brevipinnis* and the abundance of native aquatic fauna. The potential invasion of this climbing species may represent a particular threat to Murray River sites above waterfalls that were formerly fish-free (e.g., above Cudgewa Falls in Burrowa – Pine Mountain National Park, Victoria). Finally, we suggest that hydroelectric tunnels will continue to represent a translocation risk for the drainages that they artificially link.

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Do abiotic mechanisms determine interannual variability in length-at-age of juvenile Arcto-Norwegian cod?

Geir Ottersen, Kristin Helle, and Bjarte Bogstad

Abstract: For the large Arcto-Norwegian stock of cod (*Gadus morhua* L.) in the Barents Sea, year-to-year variability in growth is well documented. Here three hypotheses for the observed inverse relation between abundance and the mean length-at-age of juveniles (ages 1–4) are suggested and evaluated. Based on comprehensive data, we conclude that year-to-year differences in length-at-age are mainly determined by density-independent mechanisms during the pelagic first half year of the fishes' life. Enhanced inflow from the southwest leads to an abundant cohort at the 0-group stage being distributed farther east into colder water masses, causing lower postsettlement growth rates. We can not reject density-dependent growth effects related to variability in food rations, but our data do not suggest this to be the main mechanism. Another hypothesis suggests that lower growth rates during periods of high abundance are a result of density-dependent mechanisms causing the geographic range of juveniles to extend eastwards into colder water masses. This is rejected mainly because year-to-year differences in mean length are established by age 2, which is too early for movements over large distances.

Résumé : La variabilité inter-annuelle de la croissance chez l'important stock arcto-norvégien de Morues franches (*Gadus morhua* L.) de la mer de Barents est bien connue. On trouvera ici la proposition et l'évaluation de trois hypothèses pour expliquer la relation inverse observée chez les juvéniles (âges 1–4) entre la densité et la longueur moyenne à un âge donné. L'étude de données détaillées nous ont amenés à conclure que les différences inter-annuelles des longueurs à un âge donné sont déterminées principalement par des mécanismes indépendants de la densité durant la première demi-année pélagique de vie des poissons. Des influx accrus du sud-ouest entraînent le déplacement à l'âge 0 d'une cohorte importante vers l'est dans des masses d'eau plus froide, ce qui diminue les taux de croissance après l'établissement au fond. Il ne nous est pas possible de nier les effets dépendants de la densité de la variabilité des quantités de nourriture sur la croissance, mais nos données nous laissent croire que ce n'est pas là le mécanisme principal. Une autre hypothèse veut que les taux réduits de croissance durant les périodes de haute densité soient le résultat de mécanismes reliés à la densité qui amènent les juvéniles à étendre leur aire géographique vers les masses d'eau plus froide vers l'est. Cette hypothèse n'est pas retenue, car les différences inter-annuelles des longueurs moyennes sont déjà en place dès l'âge 2, âge trop précoce pour permettre des déplacements sur de grandes distances.

[Traduit par la Rédaction]

Introduction

Controlled experimental evidence has established that growth of fish is often inversely related to stock density. A change in the population size of a predator is assumed to affect intraspecific competition for food and thereby consumption and growth. An inverse relationship between growth and stock abundance is therefore indicative of density-dependent growth. Density-dependent growth in fish populations has been reported since early in the 20th century and further evidence has accumulated as stocks have become overexploited (e.g., Raitt 1939; Marshall and Frank 1999). However, other studies have found no significant correlation between growth and stock abundance (e.g., Daan 1974). It should also be noted that even when such a correlation is observed in field data it could be caused by a third variable, e.g., temperature acting on both stock abundance and growth (Jørgensen 1992).

Individual growth in fish depends on density-dependent factors, such as the availability of prey. However, growth is also the integration of a series of processes (feeding, assimilation, metabolism, transformation, and excretion), the rates of which are all controlled by temperature (Michalsen et al. 1998). Brander (1995) examined 17 North Atlantic cod stocks and found that most of the variability in growth among stocks could be attributed to variation in temperature.

The Arcto-Norwegian (or Northeast Arctic) cod spawn in March–May in patchy areas off northern and mid-Norway, with spawning mainly taking place in the Lofoten region (Fig. 1). Spawning occurs at the same sites each year, but spawning depth varies considerably, following the thermocline between the cold water of the Norwegian coastal current and

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G. Ottersen,[1,2] K. Helle, and B. Bogstad. Institute of Marine Research, P.O. Box 1870, Nordnes, N-5817 Bergen, Norway.

[1]Corresponding author (e-mail: geir.ottersen@bio.uio.no).
[2]Present address: University of Oslo, Department of Biology, Zoology Division, P.O. Box 1050, Blindern, N-0316 Oslo, Norway.

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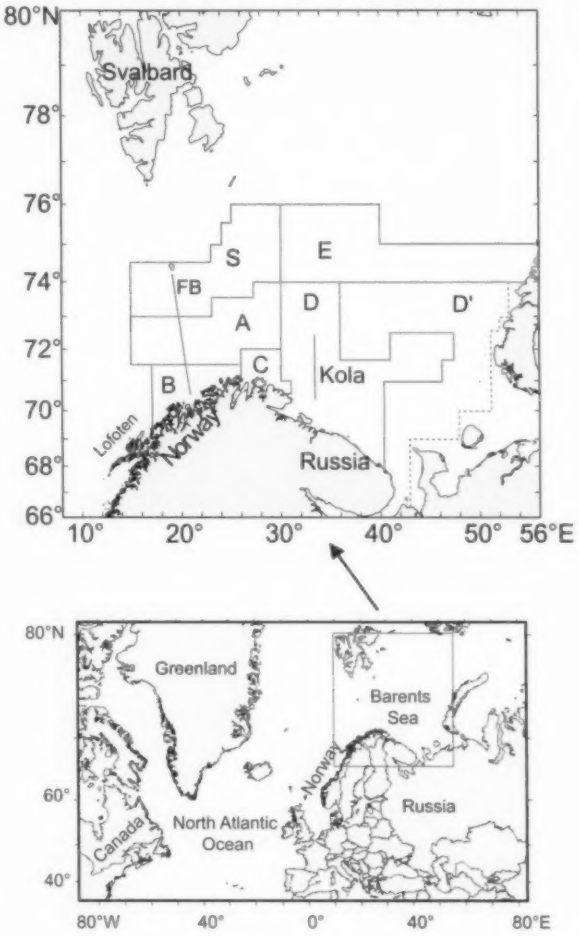
the warmer Atlantic water. Eggs and larvae are carried by the currents to the north and east into the southern Barents Sea and south and west of Svalbard (Fig. 1), 600–1200 km from their spawning ground. The spatial distribution of pelagic juveniles has substantial interannual variation. In some years, a typical westerly distribution occurs, whereas in others, an easterly distribution is observed. They settle to the bottom when they are about 5 months old. The immature cod feed at the bottom and in the midwater layers and make seasonal east–west and north–south migrations. The ranges of these migrations increase with age, and at an age of 3–4 years, when capelin (*Mallotus villosus*) become a major food item, cod follow the spawning migration of capelin to the coasts of northern Norway and Murman (Nakken 1994).

Year-to-year variability in the growth of Arcto-Norwegian cod has been observed over a long period (Nakken 1994; Michalsen et al. 1998). Several authors have suggested that this variability in growth is density dependent (e.g., Rollefsen 1953; Nilssen et al. 1994). On the other hand, Jørgensen (1992) found an apparent absence of density-dependent growth because no significant relationship between length increments and stock size could be established. Nakken and Raknes (1987) noted a negative correlation between abundance and mean length of cod but concluded that density-dependent growth was unlikely. At the 0-group stage (5 months old), a positive relationship between abundance and mean length, which may be explained as size-dependent survival, was shown by Ottersen and Loeng (2000).

A number of earlier authors have observed larger mean lengths-at-age for Arcto-Norwegian cod in warmer years than in colder years and have proposed a positive connection between temperature and growth (Dementyeva and Mankevich 1965; Nakken and Raknes 1987; Michalsen et al. 1998). In the Barents Sea, the water temperature decreases from west to east. This east–west gradient is a general feature regardless of interannual variability. Year-classes with an easterly distribution will consequently experience lower ambient temperatures than those with a more westerly pattern. Ottersen et al. (1998) suggest a range expansion towards the east during periods of high density, apparently independent of the temperature conditions. Thus, high abundance leads to low ambient temperatures, which should cause reduced growth rates (Helle et al. 2000).

Temperature variability in the Barents Sea is, to a large degree, caused by fluctuations in the inflow of warm Atlantic water masses from the southwest (Ådlandsvik and Loeng 1991). A year with enhanced inflow of Atlantic water masses provides the 0-group cod with a relatively warm and prey rich environment (Helle and Pennington 1999) and carries them farther east in the Barents Sea (Ottersen et al. 1998). It is well documented that years with positive temperature anomalies tend to produce cohorts of high abundance, both as 0-group and as age-3 recruits (Sætersdal and Loeng 1987; Ottersen and Sundby 1995). Thus the distribution of cohorts with high abundance at the 0-group stage is extended farther into the colder eastern parts of the Barents Sea (Helle et al. 2000). Several Russian studies (e.g., Maslov 1960) have shown that 1-year-old cod remain close to where they settled. Thus an easterly 0-group distribution would lead to an easterly distribution of the age-1 group, which could influence the distribution of the year-class at older ages. Furthermore, one would expect lower growth rates for these year-classes, leading to reduced juvenile length-at-age, on average.

Fig. 1. The Barents Sea with subareas framed. For analysis by subarea, we only use A, B, C, and D covered each year throughout the full period 1983–2000. The Fugløya – Bear Island section, where the inflow has been estimated (FB), and the Kola section, where temperatures have been measured (Kola), are shown. The general location of the region is displayed in the lower map.



Here we examine the possible connections, described above, between abundance and mean length-at-age of immature Arcto-Norwegian cod. To summarise, the following three hypotheses are investigated. (H1) Year-to-year differences in length-at-age of demersal, immature Arcto-Norwegian cod are determined by population size, high abundance leading to reduced growth (density-dependent growth). (H2) Year-to-year differences in mean length-at-age of demersal, immature Arcto-Norwegian cod are determined by abundance influencing growth through altering geographical distribution patterns. Lower growth rates during periods of high abundance are a result of density-dependent mechanisms causing the geographic range of the population to extend eastwards into colder water masses, causing a de-

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crease in the mean ambient temperature. (H3) Year-to-year differences in mean length-at-age of demersal, immature Arcto-Norwegian cod are determined by abiotic, density-independent mechanisms during the pelagic stages. Enhanced inflow of warm, prey-rich Atlantic water masses from the southwest leads to an abundant cohort as 0-group that is distributed farther east into colder water masses, which causes lower average postsettlement growth rates.

Material and methods

The data on length and abundance (ages 1–4) of Arcto-Norwegian cod are from the Institute of Marine Research bottom trawl surveys conducted in the Barents Sea each January–March since 1981 (abundance data for 1981 are considered unreliable and not used). For a detailed description of the survey and its history, see Jakobsen et al. (1997).

A Campelen 1800 shrimp trawl with mesh size of 80-mm in front was used until 1993, with a codend of 35- to 40-mm stretched mesh size. Because this mesh can cause considerable size selection of 1-year-old cod, the mesh size was reduced to 22 mm in 1994. The survey indices have not been corrected for this change.

The surveys followed a stratified random design until 1990 and a stratified systematic design from 1991 onwards. Until 1992, the area covered by the survey was limited to the ABCD region (Fig. 1). The number of trawl stations varied, but the entire ABCD area was always reasonably well covered. We have used established area definitions, where the subareas are based on the observed fish distribution, the important fishing grounds, and the statistical areas for recording commercial catches. The estimates of mean length and swept area estimates of abundance for the whole Barents Sea are taken from ICES (2001), with slight adjustments for age 3 and for 1981–1982, which are based on recent recalculations.

We examine data on length and abundance by age for the entire Barents Sea and for each of the subareas A, B, C, and D (numerical values available from the authors). Because area-specific data are scanty for the younger age classes in 1981 and 1982, the analysis is based on 1983 and onwards. Abundance and mean length estimates by area are in the annual survey reports, but those estimates differ somewhat from the recalculated values (consistent with the values for the entire area) given here. Typical values of the standard deviation of length-at-age for the total area are 2 cm for age 1, 3 cm for age 2, and 4 cm for ages 3 and 4. The estimation procedure for age-based abundance indices and mean lengths-at-age is given in Appendix A.

The time series was revised to account for several changes in methodology (Jakobsen et al. 1997). However, no correction has been made for the introduction of an inner net in 1994. This mainly affects the abundance and mean length estimates of age-1 fish, whose abundance index before 1994 will be underestimated and mean length before 1994 will be overestimated. For this reason, the results for ages 2 and 3 are more reliable than those for age 1. Furthermore, the mean length-at-age estimates for 1988 and earlier years are slight overestimates, which is due to a change from bobbins to rock-hopper gear in 1989 being accounted for in the calculation of abundance-at-age but not in the calculation of mean length-at-age. See Jakobsen et al. (1997) for a more thorough description and references.

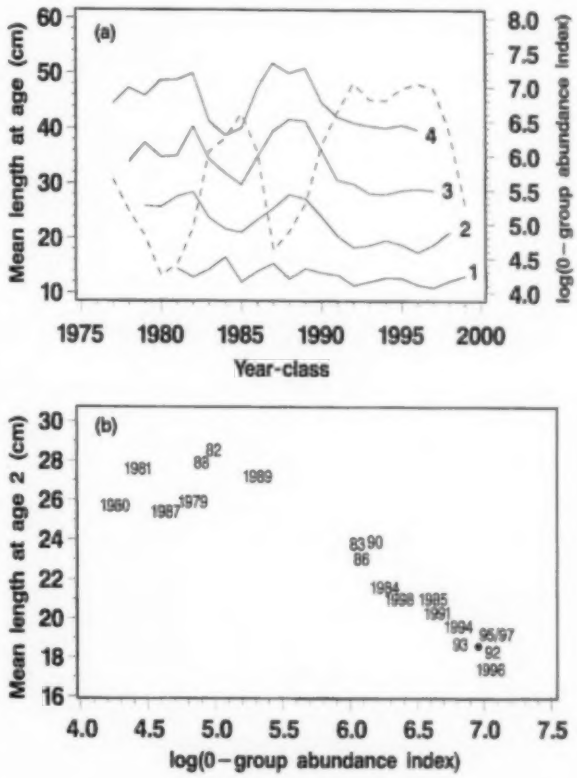
To examine interannual fluctuations within each of the

Table 1. Correlations between abundance at ages 0–4 (logarithmically transformed) and mean length at ages 1–4 for the 1978–2000 year-classes of Arcto-Norwegian cod.

	Length-at-age			
Abundance-at-age	1	2	3	4
0-group	–0.54*	–0.93***	–0.81***	–0.87***
1	–0.57**	–0.71**	–0.64**	–0.71**
2		–0.62**	–0.50**	–0.73**
3			–0.51*	–0.68**
4				–0.41

Note: The 1978 year-class was age 4 in 1982, the first year with reliable abundance estimates. Significance levels: *, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$.

Fig. 2. (*a*) Temporal development of length (in cm) for ages 1 to 4 (solid lines) and abundance as 0-group (natural logarithmic transform of the index corrected for trend by Nakken and Raknes (1996), broken line) of Arcto-Norwegian cod year-classes from 1977 to 1999 (but note that year-classes covered necessarily varies with age). (*b*) Scatter plot showing abundance as 0-group (log-transformed) versus mean length at age 2 for Arcto-Norwegian cod year-classes 1979–1998. When it is difficult to distinguish between different years, only the last two digits are shown in the graph; when the values for 2 years coincide, a dot is used.



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subareas A, B, C, and D relative to the entire region, we defined relative abundance for a specific age group and year as the number within a particular area divided by the total number of this age group within ABCD. This allows for examination of possible shifts in abundance among areas and their relationship to overall abundance. A direct comparison between abundance within the whole of ABCD and that within each area would be misleading because most of the fish studied are typically found in area D. Thus fluctuations in area D would dominate the total variability.

In addition to bottom trawling, the winter survey also acoustically measures fish density and records temperature (conductivity–temperature–depth (CTD)/Rosette) profiles. We use the centre of mass of the spatial distribution (in degrees longitude) by age based on the acoustic measurements for the years 1988–1995 as a supplementary measure of horizontal location (Ottersen et al. 1998).

Ambient (fish-density weighted) winter temperature means for each age group for the years 1985–1999 were calculated from bottom temperatures and the swept area data described above. To extend our time series backwards, we also use ambient winter temperatures for the years 1978–1984 from Nakken and Raknes (1987), although these were based on acoustic densities. However, Ottersen et al. (1998) compared the variation of cod density in space and time from swept area and acoustic density estimates and concluded that the main developments were similar.

Abundance indices for pelagic 0-group cod have been worked out from material gathered during the International Barents Sea 0-group surveys, which are conducted in late August to early September. The 1974–1999 indices of abundance used in this paper were adjusted to take into account trends in catchability (Nakken and Raknes 1996), with post-1995 values taken from ICES (2001). Estimates for the years 1989–1996 of ambient temperature for 0-group cod are from Stensholt and Nakken (1998).

Estimates of monthly atmospherically driven water flux through the Fugløya–Bear Island section (FB, Fig. 1) for the period 1970–1999 are based on a numerical hydrodynamic model (Ådlandsvik and Loeng 1991; Loeng et al. 1992; B. Ådlandsvik, Institute of Marine Research (IMR), Bergen, Norway, personal communication). We used winter (January–April) means (F_{FB}) in this paper. Density-driven (baroclinic) currents are not included in the model; hence these flux measurements only reflect the variability in atmospheric forcing (barotropic component). The mean F_{FB} for the period studied is small compared with estimates of total transport, but the variability in total inflow is mainly due to year-to-year differences in barotropic conditions, as reflected in F_{FB} (Ottersen and Stenseth 2001).

Observations of the sea temperature in the Kola meridian transect (33°30′E, 70°30′N to 72°30′N), which intersects the Murman Current in the south central Barents Sea (Fig. 1), are considered good indicators of thermal conditions in the entire Barents Sea region.

Monthly means have been calculated by averaging along the transect and vertically from water depths of 0 to 200 m. We used 1975–1999 winter (December previous year – February) means, T_{Kola}, with the pre-1995 data taken from Bochkov (1982) and Tereshchenko (1996), whereas PINRO, Murmansk, provided the 1995–1999 values.

Table 2. Correlations between abundance (logarithmically transformed) and centre of mass of winter distribution (in east–west direction, degrees longitude) in 1988–1995 for Arcto-Norwegian cod at ages 0–3.

	Centre of mass of distribution at age		
Abundance-at-age	1	2	3
0-group	0.66	0.79*	0.41
1	0.43	0.77**	0.50
2		0.91**	0.63
3			0.79*
4			

Note: Significance levels: *, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$. No correlations involving age 4 were statistically significant.

Standard Pearson product-moment correlations were used to study linear covariation. However, correlation analysis depends on assumptions that are rarely met by time series data. In our case, as often occurs in biology, we have short, positively autocorrelated time series. This violates the assumption of statistical independence of the points in the time series used in estimating standard significance levels. As a result, the significance levels calculated by standard statistics packages are inflated compared with the true significance level. To deal with this, we estimated the effective number of independent observations (N_e), adjusted for order 1 and order 2 autocorrelations, using the formula of Quenouille (1952): $N_e = N/(1 + 2r_{a1}r_{b1} + 2r_{a2}r_{b2})$. Here N is the number of data points common to the two series, r_{a1} and r_{b1} are the lag-one autocorrelations, and r_{a2} and r_{b2} the lag-two autocorrelations for the time series a and b.

Results

Mean lengths of Arcto-Norwegian cod at ages 1–4 are negatively correlated with the total abundance of the cohort at the same and earlier ages (Table 1, Fig. 2). For the years 1988–1995, the abundance of a cohort and centre of mass of the spatial distribution at ages 1–4 are positively correlated; high abundance at age a is linked to an easterly displacement at age a and at age $a + 1$ (Table 2). Fish in area A were on average 1.2 cm longer than in area D at age 2, 2.5 cm longer at age 3, and 1.7 cm longer at age 4. The east–west differences in length were not significantly correlated (at the 5% level) with abundance of the age group within ABCD.

The observed size-at-age is generally lower in the period 1994–2000 (when an inner net was used) than in the earlier years. To determine whether this is due to a smaller mean size-at-age or to possible differences introduced by the gear change, we compared the size-at-age from the Russian bottom trawl survey in autumn (data given in ICES (2001)) with the Norwegian data for the periods 1985–1993 and 1994–2000. The results of this comparison are shown in Table 3. The size-at-age is larger in the first period in both surveys, confirming that the difference was not an artifact introduced by changes in Norwegian survey methodology.

The correlations between the relative abundance of age-2 cod in each subarea and in ABCD are given in Table 4. The temporal development of abundance of age-2 fish in ABCD is compared with the fractions in A and D (Fig. 3). The relative abundance in area A fluctuates out of phase with the

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Table 3. Mean length-at-age (cm) for the periods 1985–1993 and 1994–2000 for the Norwegian (Nor) and Russian (Rus) surveys.

	Age 1		Age 2		Age 3		Age 4	
Period	Nor	Rus	Nor	Rus	Nor	Rus	Nor	Rus
1985–1993	13.3	13.7	23.8	21.3	36.4	29.8	46.4	41.2
1994–2000	12.1	11.3	18.9	19.2	29.0	28.0	41.2	36.8

Note: Russian surveys, which are conducted in late autumn, are shifted 1 year and 1 age for comparative purposes. Age 1 in 1988 and 1990 are omitted from the calculation because there are no Russian data available.

Table 4. Correlations between abundance of Arcto-Norwegian cod at age 2 in the ABCD region (logarithmically transformed) and relative abundances in the subareas as well as between the subareas for the years 1983–2000.

	Abundance in ABCD	Fraction in B	Fraction in C	Fraction in D
Fraction in A	–0.45*	0.50**	0.56**	–0.95***
Fraction in B	–0.73***		0.71***	–0.70***
Fraction in C	–0.79***			–0.79***
Fraction in D	0.65***			

Note: Significance levels: *, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$.

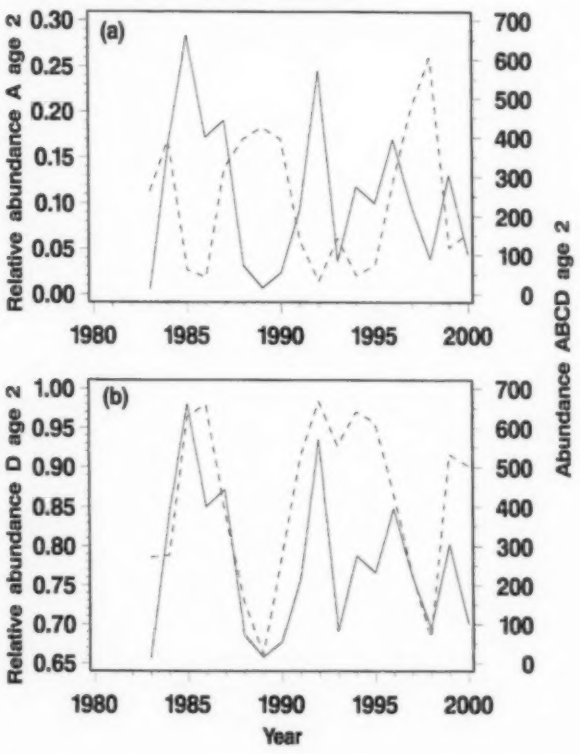

Fig. 3. Temporal development in 1983–2000 of abundance of age-2 Arcto-Norwegian cod in the ABCD area (numbers in millions, solid line) compared with relative abundance (fraction) in (*a*) area A and (*b*) area D, broken lines.

overall abundance, which is in synchrony with the fraction in area D.

T_{Kola} has fluctuated more or less in accordance with F_{FB} during the study period (Fig. 4). F_{FB} in the winter preceding spawning is positively correlated with cod abundance at the 0-group stage and with an easterly distribution. Furthermore, enhanced winter inflow preceding spawning is negatively correlated with ambient temperatures and length-at-age for ages 1–4 (Table 5; Fig. 5).

Discussion

This study demonstrates that the mean length of 1- to 4-year-old Arcto-Norwegian cod tends to be small when the abundance of the same cohort and (or) neighbouring cohorts is high. Three hypotheses are put forth, each giving an alternative explanation to the observed pattern. The main focus of the discussion is to disentangle the evidence available from our own and earlier studies and to draw a conclusion regarding the validity of the suggested mechanisms.

Nilssen et al. (1994) analysed weight-related specific growth rates and abundance-at-age data for Arcto-Norwegian cod during the period 1951–1991. They found a negative correlation and concluded that density-dependent growth was clearly demonstrated. On the other hand, Jørgensen (1992), based on data for nearly the same period (1953–1989), was not able to detect density-dependent growth for ages 1 through 8. Nakken and Raknes (1987) noted a negative relationship between abundance and mean length of 4- to 6-year-old cod for the period 1978–1984. However, because of the stock size in this period amounting to only a minor fraction of the long-term average, they found it hard to accept that density-dependent growth occurred in those years. The main difference between the study of Nilssen et al. (1994) and the other two is that the former studied weight rather than length. We use length-at-age data because weight data for 1981–1984 and 1987 are missing from our time series, and the older weight data are generally considered less reliable than the data on length.

Density-dependent growth is typically assumed to be related to high stock abundance leading to a reduction in individual growth rates (H1) as a consequence of reduced food rations. Therefore, data on prey abundance should be taken into account in a discussion of density-dependent growth. The main prey species of age-1 cod are krill, amphipods,

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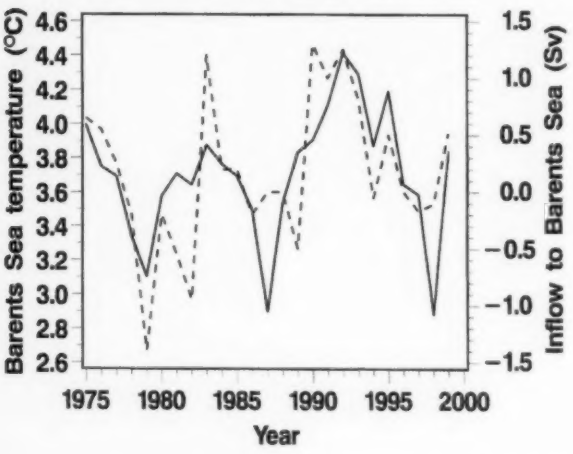
Fig. 4. Winter inflow to the Barents Sea from the southwest (solid line, modelled atmospherically driven water volume flux through the Fugløya – Bear Island section) and Barents Sea winter temperature (broken line, 0- to 200-m depth mean values from the Kola meridian transect) in 1974–1999.



and capelin; capelin increases in importance with increasing predator age (Bogstad and Mehl 1997). The abundance of capelin has varied greatly during the period of this study (Gjøsæter 1997) as has the abundance of zooplankton, such as amphipods and krill (Dalpadado et al. 2002). A more detailed study involving the abundance of zooplankton in the sea as well as in cod stomachs is needed to determine whether growth of 1-year-old cod is density dependent.

Our data show clear negative correlations between abundance and length-at-age. However, neither this nor earlier studies give us sufficient evidence to conclude that density dependence (H1) is the dominant mechanism regulating the growth of Barents Sea cod.

This led us to pursue the idea that abundance affects growth through altering geographical distribution patterns and ambient temperatures of 1- to 4-year-old fish (H2). Myers and Stokes (1989) identified three ways in which the geographical distribution of a fish population may change in response to changes in overall population size: (*i*) a proportional increase throughout its range; (*ii*) a range extension in which the population increases relatively more in marginal habitats; or (*iii*) a relatively greater increase in the prime habitat, i.e., increased population density leads to higher concentrations. A fourth possible response is a shift in the region of greatest density.

Positive correlations between abundance and easterly centre of mass of spatial distribution indicates that at high abundances the juvenile cod either extend their distribution farther east or the region of greatest density is shifted eastwards. This is further supported by the total abundance in ABCD being positively correlated with the fraction of age-2 cod in the easterly area D, while being negatively correlated with the fractions in A, B, and C. The relative abundance seems to fluctuate in phase in these three areas and out of phase with area D.

In principle, slight movements could result in significant differences in the number of fish in one area versus another

Table 5. Correlations between atmospherically driven winter inflow to the southwestern Barents Sea (F_{FB}) and sea temperature (T_{Kola}), abundance of 0-group cod ($N_{0\text{-group}}$), geographical distribution (east–west displacement, Lon_i), ambient temperatures (T_i), and average length-at-age ($Length_i$), respectively, all for age groups 1–4.

	r	n
T_{Kola}	0.63***	26
$N_{0\text{-group}}$	0.43*	26
Lon_1	0.57	8
Lon_2	0.84*	8
Lon_3	0.75	8
Lon_4	0.38	8
T_0	–0.50	8
T_1	–0.57**	22
T_2	–0.47*	23
T_3	–0.56**	23
T_4	–0.74***	23
$Length_1$	–0.21	19
$Length_2$	–0.41	20
$Length_3$	–0.53**	20
$Length_4$	–0.60**	20

Note: The time series are for winter except 0-group data which are autumn. All correlations are for inflow in the year spawned. Data from 1974 (first year-class with any data on juvenile fish) or whenever available. Significance levels: *, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$.

if there are many fish distributed in the vicinity of the border between two areas. However, although the fish are not evenly distributed, there is no evidence for such an effect being important. Interannual differences in the centre of mass of spatial distribution are pronounced, particularly for ages 1–3, and the shifts are far larger than those that would be caused by fish simply crossing a border. Furthermore, distribution maps of cod density during the winter surveys give no indication of higher than average concentrations along the borders between areas (maps for 1990 and 1994 given in Ottersen et al. (1998), other unpublished maps available from the authors).

Bioenergetic considerations suggest that temperature selection by fish should be density dependent. When rations are limited, the temperature yielding the highest growth rate decreases as ration size decreases (Elliott 1975). Therefore, if fish select the habitat that maximises their growth rate, preferred temperatures should decrease as food supply decreases (Elliott 1975; Swain and Kramer 1995). If food is a limiting factor, then the proportion of the population occupying habitats of lower temperature should increase with population size (Swain and Kramer 1995).

Because a more easterly distribution in the Barents Sea implies lower ambient temperatures (Ottersen et al. 1998), the observed spatial shifts could be taken as field support for the above argument. That is, a density-dependent decrease in food availability for individual fish should result in increased preference for habitats with lower density-independent costs, in this case lower temperatures.

The mechanisms suggested above imply that fish actively move in response to variation in population density. Al-

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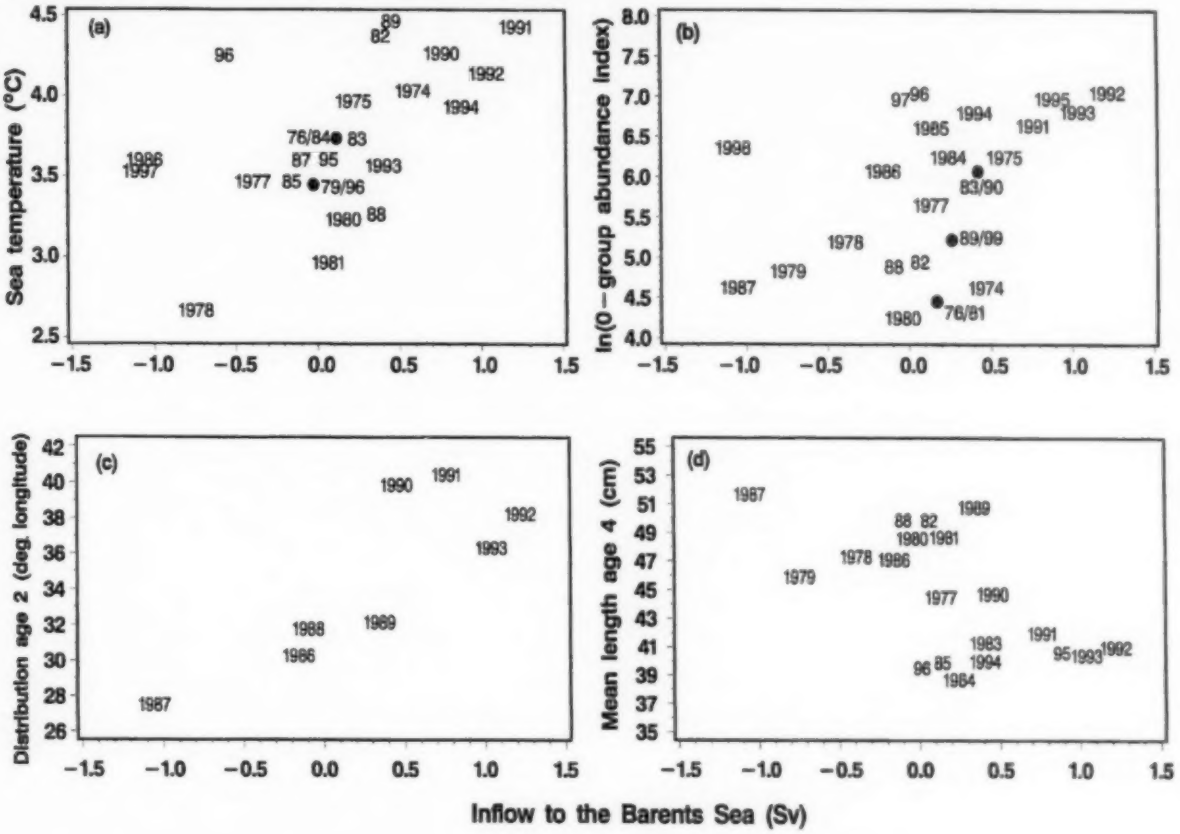
Fig. 5. Winter inflow to the Barents Sea from the southwest (modelled atmospherically driven water volume flux through the Fugløya – Bear Island section) plotted against (*a*) Barents Sea (Kola section) temperature, (*b*) abundance of Arcto-Norwegian cod as 0-group (natural logarithmic transform of the index corrected for trend by Nakken and Raknes 1996), (*c*) centre of distribution of cod age 2 (degrees longitude), and (*d*) mean length of cod age 4. Note that in (*c*) and (*d*), year-class, not year, is shown so that all variables are related to inflow in the winter preceding spawning. Data from 1974 (first year-class with any data on juvenile fish, i.e., ambient temperature at age 4) or whenever available. When it is difficult to distinguish between different years, only the last two digits are shown in the graph; when the values for 2 years coincide, a dot is used.



though our results (and those of Kristiansen et al. 2001) indicate easterly shifts in periods of high abundance, we are not able to rule out other causes for the apparent movement. Spatial variation in natural mortality, e.g., cannibalism, may shift the centre of mass without any active habitat selection being involved (Bogstad et al. 1994). The variability in the mortality rates of Arcto-Norwegian cod of ages 0–3 caused by cannibalism is large, as indicated by ICES (2001).

The fact that undermines much of the foundation for H2 is, however, that year-to-year differences in length seem to be established by age 2 (as demonstrated also by Jørgensen (1992) and Michalsen et al. (1998)). The large spatial movements necessary by these young cod if H2 is true seriously questions the validity of this hypothesis. This is especially the case as age-1 cod tend to remain in the area where they settled during autumn as 0-group (Maslov 1960) and seasonal migrations by 2-year-old cod seem to be small (Ottersen et al. 1998).

We therefore considered mechanisms occurring during the first 1–2 years of life that may affect year-to-year differences in length-at-age. It is natural to look to the environment to find sources for variability established at an early age (see Introduction). Thus, seemingly density-dependent growth may be caused by density-independent environmental effects during the early life stages, which determine the distribution patterns and indirectly, growth, as proposed in H3.

The following shows how our new results, supported by earlier authors, give evidence in support of H3. The fact that F_{FB} and T_{Kola} fluctuate in synchrony indicates that enhanced inflow of Atlantic water masses from the southwest is related to generally higher Barents Sea temperatures as earlier suggested by Ådlandsvik and Loeng (1991). We have shown that there is a positive correlation between inflow and the abundance of 0-group cod, a similar relationship between sea temperature and year-class strength at the 0-group stage has been described earlier by Ottersen and Sundby (1995) and others. The positive correlation between F_{FB} and easterly centre of mass at ages 1–4, and the negative correlation with ambient temperature at ages 0–4, shows that cod are distributed farther into the colder water masses of the eastern

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Barents Sea during years with high inflow. Finally, F_{FB} is negatively correlated with lengths-at-age, indicating that the above mechanisms lead to lower postsettlement growth rates. We conclude that this chain of events, summarised in H3, gives the most consistent explanation of our observations.

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Appendix A

Length-based abundance indices by area are estimated as follows. For each trawl station and 1-cm length group, a point observation of fish density is calculated as

$$\rho_{s,i} = \frac{f_{s,i}}{a_{s,i}}$$

where $\rho_{s,i}$ represents the density of fish of length i observed at station s, $f_{s,i}$ is the raised length frequency, and $a_{s,i}$ is the swept area ($d_s \mathrm{EW}_i$, where d_s is the towed distance and EW_i is the length-dependent effective fishing width).

Point estimates of density at length are summed up to 5-cm length groups $\rho_{s,l}$, where l denotes 5-cm length groups. Stratified indices of abundance by length groups and strata $N_{p,l}$ are calculated as

$$N_{p,l} = \frac{A_p}{S_p} \sum_{s=1}^{S_p} \rho_{s,l}$$

where A_p is the area of stratum p (or the part covered by the survey) and S_p is the number of stations in stratum p (23 strata presently used).

Before 1984, a representative subsample of 100 fish per station was age determined at two stations per strata. From 1984, a stratified sample design was used, with samples of five fish per 5-cm length group at each station. In 1993–1995, two fish were sampled per 5-cm length group, and the number of stations sampled per stratum was increased from two to four. Before 1996, the strata structures used were different from the present 23-strata system. Since 1996, one fish is sampled per 5-cm length group at all predetermined bottom trawl stations with more than 10 specimens of cod in the catch. For each 5-cm length group, age–length keys are used to estimate the proportions at age. All age samples from the same stratum and length group are given equal weight:

$$w_{p,l} = \frac{N_{p,l}}{n_{p,l}}$$

where $n_{p,l}$ is the number of age samples in stratum p and length group l and the proportions of fish of various ages in a given length group are estimated by

$$P_a^{(l)} = \frac{\sum_p n_{p,a,l} w_{p,l}}{\sum_p n_{p,l} w_{p,l}}$$

where $P_a^{(l)}$ is the weighted proportion of age a in length group l and stratum p and $n_{p,a,l}$ is the number of age samples of age a in length group l and stratum p.

The numbers-at-age are then calculated as

$$N_a = \sum_p \sum_l N_{p,l} P_a^{(l)}$$

and the mean length-at-age is calculated as

$$L_a = \frac{\sum_p \sum_l \sum_j L_{a,p,l,j} w_{p,l}}{\sum_p \sum_l \sum_j w_{p,l}}$$

where $L_{a,p,l,j}$ is the mean length of sample j in length group l, stratum p, and age a.

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Historical changes in the distribution and functions of large wood in Puget Lowland rivers

Brian D. Collins, David R. Montgomery, and Andrew D. Haas

Abstract: We examined changes in wood abundance and functions in Puget Lowland rivers from the last ~150 years of land use by comparing field data from an 11-km-long protected reach of the Nisqually River with field data from the Snohomish and Stillaguamish rivers and with archival data from several Puget Lowland rivers. Current wood abundance is one to two orders of magnitude less than before European settlement in the Snohomish and Stillaguamish basins. Most importantly, wood jams are now rare because of a lack of very large wood that can function as key pieces and low rates of wood recruitment. These changes in wood abundance and size appear to have fundamentally changed the morphology, dynamics, and habitat abundance and characteristics of lowland rivers across scales from channel unit to valley bottom. Based on our field studies, rivers had substantially more and deeper pools historically. Archival data and field studies indicate that wood jams were integral to creating and maintaining a dynamic, anastomosing river pattern with numerous floodplain channels and abundant edge habitat and routed floodwaters and sediment onto floodplains. Establishing the condition of the riverine landscape before European settlement sets a reference against which to evaluate contemporary conditions and develop restoration objectives.

Résumé : La comparaison d'observations de terrain sur un tronçon protégé de 11 km de la rivière Nisqually à des données provenant des rivières Snohomish et Stillaguamish, ainsi qu'à des données anciennes sur plusieurs rivières des terres basses de Puget nous ont permis d'étudier les changements dans l'abondance et le rôle du bois dans ces rivières au cours d'approximativement les dernières 150 années d'utilisation des terres. L'abondance actuelle du bois est de 10 à 100 fois moins grande qu'avant la colonisation des bassins de la Snohomish et de la Stillaguamish par les européens. Ce qui est plus important, c'est la rareté des embâcles à cause de l'absence de très grandes pièces de bois pour servir de structures maîtresses et des taux réduits d'apport de bois. Ces changements dans l'abondance et la taille du bois semblent avoir modifié de façon fondamentale la morphologie, la dynamique, ainsi que la densité et les caractéristiques des habitats dans les rivières des terres basses à diverses échelles, depuis les unités individuelles de lit de rivière jusqu'à l'ensemble du fond de la vallée. D'après nos observations de terrain, les rivières avaient des cuvettes plus nombreuses et plus profondes dans le passé. Nos données d'archives et nos études récentes sur le terrain indiquent que les embâcles jouaient un rôle essentiel dans la création et le maintien d'une structure fluviale dynamique et anastomosée, caractérisée par de multiples chenaux sur la plaine d'inondation et de nombreux habitats de lisière, de même que dans le déversement des eaux de crue et des sédiments sur la plaine d'inondation. L'établissement des conditions du paysage fluvial existant avant la colonisation européenne fournit un point de référence pour évaluer la situation présente et fixer les objectifs de restauration.

[Traduit par la Rédaction]

Introduction

Numerous studies have established that wood has a primary influence on channel form in small forest streams, but less is known about wood in larger rivers. Recent literature summaries indicate that the influence of wood "decreases with increasing channel size" (Bilby and Bisson 1998). However, in large rivers of the Pacific Northwest, 19th and 20th century stream cleaning greatly diminished wood abundance, and riparian forest clearing and levee construction reduced the potential for lowland floodplain rivers to recruit wood (e.g., Sedell and Luchessa 1981; Sedell and Frogatt 1984). This suggests that the current condition of regional rivers is not representative of their historical condition and highlights the need for a benchmark against which to assess restoration efforts. Recent work on the Hoh and Queets rivers in Washington's Olympic National Park (Sedell et al. 1984; Abbe and Montgomery 1996) shows how wood shapes large channels and their aquatic habitat. The purposes of this paper are (*i*) to document historical conditions in

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B.D. Collins[1] **and D.R. Montgomery.** Department of Earth and Space Sciences, University of Washington, Seattle, WA 98195, U.S.A.
A.D. Haas.[2] Tulalip Tribes Natural Resources Department, 7615 Totem Beach Road, Marysville, WA 98271, U.S.A.

[1]Corresponding author (e-mail: bcollins@u.washington.edu).

[2]Present address: Snohomish County Department of Public Works, Surface Water Management Division, 2731 Wetmore Avenue, Suite 300, Everett, WA 98201, U.S.A.

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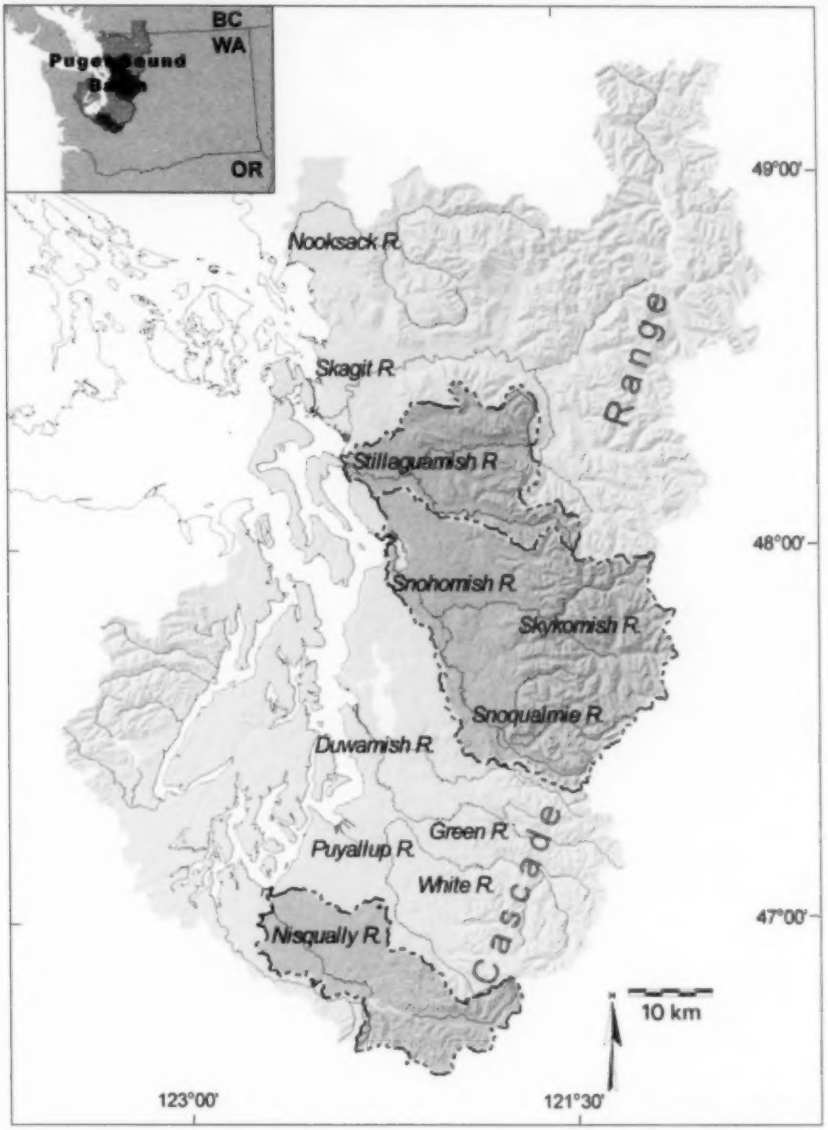
Fig. 1. Locations of Puget Lowland rivers and study sites on the Nisqually, Snohomish, and Stillaguamish Rivers, Washington State, U.S.A.



Puget Sound rivers, to compare to current conditions documented in the Olympic Peninsula studies for assessing these studies' geographic generality, and to extend those studies by evaluating a broader range of wood structures and effects; (*ii*) to document changes that have occurred since European settlement; and (*iii*) to use these changes as a long-term removal experiment and to evaluate hypotheses about wood functions. We do this by comparing the role of wood in Puget Lowland rivers under current and historical conditions by assessing amounts and types of wood accumulations, and their influence on channel structure, in relatively developed and undeveloped rivers using both field and archival studies.

To investigate representative current conditions, we examined the Snohomish and Stillaguamish rivers. In the past ~150 years, both rivers have been snagged, leveed, and isolated from their floodplains, which have been drained, ditched, and converted to agriculture and other uses. Their land-use history and current condition is typical of other rivers in the region (Sedell and Luchessa 1981).

We took two approaches to investigating the historical condition of Puget Sound rivers. We collected field data from a reach of the Nisqually River (Fig. 1) that has been protected from development in the 20th century on the assumption that it has retained characteristics of the pre-European-settlement condition. The river has natural banks, and logging of the valley-bottom forest was limited to the larger conifers late in the 19th century. There are no other undeveloped rivers in the Puget Sound region, which limits the extent to which it can be inferred that the Nisqually

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River represents historical conditions. To provide context for interpreting the regional significance of the Nisqually data, we also use archival sources, including annual reports of the Army Engineers (Annual Reports of the Chief of Engineers, U.S. War Department, 1875–1980; hereafter referenced as U.S. War Department), settlers' accounts, maps and reports by the U.S. Coast and Geodetic Survey, U.S. General Land Office, U.S. Department of Agriculture, U.S. Geological Survey (USGS), and other agencies. Archival and process studies are complementary; field studies provide detail on and generate hypotheses about physical processes, whereas archival studies help characterize regional variation (which is not possible from field studies when the number of field sites is limited) and the applicability of hypotheses generated from field studies (Collins and Montgomery 2001). Additionally, archival studies can describe processes and features at the river-valley scale that may no longer be present owing to human changes to the landscape.

Study areas

The Nisqually River begins at the terminus of the Nisqually Glacier in Mount Rainier National Park (Fig. 1) and drains an 1890 km^2 basin. The LaGrande and Alder hydroelectric projects are at river kilometres 68 and 71, respectively (Ames and Bucknell 1981). The dams are not operated for flood control, but reduce some flood peaks. Below these dams, the Nisqually River passes through a mix of agricultural, forested, and rural land and then traverses or borders the Fort Lewis military reservation, between river kilometres 4 and 31. (River kilometres are from USGS topographic maps or Ames and Bucknell (1981).)

The study reach is between river kilometres 7.4 and 18.7, above tidal influence, where the river has incised a valley ~70 m deep into the land surface since the last glaciation, which ended ~14 000 years ago. The forested valley bottom is between 0.6 km and 1.1 km wide and includes numerous floodplain channels. Bankfull channel width in 1999 averaged 100 m, and gradient ranges between 0.002 and 0.005. The mean annual discharge between 1948 and 1999 was 37.3 $m^3 \cdot s^{-1}$ at a gage at river kilometre 35.1. The largest recorded flood, on 8 February 1996, was estimated as 1416 $m^3 \cdot s^{-1}$ (USGS 1997).

The reach is unique in the Puget Lowland in having a mature valley-bottom forest and no artificial levees or bank protection because it has been protected within the Nisqually Indian Reservation and Fort Lewis. The Nisqually Indians ceded most of their land to the federal government in the 1854 Treaty of Medicine Creek, but they retained rights to a reservation that included most of the study area. Although other Puget Lowland riparian forests had been cleared by the end of the 19th century, the Nisqually was not (Plummer et al. 1902) other than selective logging of conifers (primarily *Thuja plicata* (western red cedar) and secondarily *Pseudotsuga menziesii* (Douglas fir)), which were cut into bolts and floated downstream to a shake mill (George Walter, Nisqually Indian Tribe, 12501 Yelm Hwy. SE, Olympia, WA 98513, U.S.A., personal communication). In 1917, the United States annexed 3370 acres of the Nisqually Indian Reservation for Fort Lewis, including the north side of the valley. In the most downstream 1 km of the study reach on private land downstream of the reservation, some forest was converted to agricultural and residential uses before 1910 (Mangum 1911). Elsewhere, two fish hatcheries operated by the Nisqually Tribe are the only substantial developments.

The Skykomish and Snoqualmie rivers join to form the Snohomish River, which flows westward 34 river kilometres to Possession Sound at the city of Everett (Fig. 1). The river gradient declines downstream from 0.0004 to 0.00006. It splits into several major sloughs (Steamboat, Ebey, and Union sloughs) and the main river, which is dredged to maintain its navigability in its lowest approximately 5 km. Because of the river's low gradient, tidal backwater extends to about river kilometre 29.

The North and South forks of the Stillaguamish River (1770 km^2) join at Arlington, at river kilometre 29. From there, it flows westward to Port Susan and Skagit Bay. The Stillaguamish is steeper than the Snohomish; its gradient declines from 0.0009 to 0.0003. The Stillaguamish splits into two channels between kilometres 18 and 10, the approximate upper limit of tidal influence. It then flows into Port Susan through Hat Slough. Before ~1906, the river flowed instead northwest through what is now a minor tidal slough (U.S. Army Corps of Engineers 1929). Levees and bank armoring are extensive on the Stillaguamish and Snohomish rivers, and agriculture dominates both valley bottoms. Riparian forests are generally either absent or narrow and dominantly small deciduous trees.

Methods

In-channel wood

To determine the abundance of wood and its location in the channel, we inventoried wood and wood jams in the Nisqually River in September–October 1998. We noted all wood greater than 0.15 m in diameter and 2 m in length and having any portion within the bankfull limits of the active channel. We recorded whether wood was on a bank, bar, low-flow channel margin, or low-flow channel middle. We measured dimensions (i.e., width, length, thickness, and height) of 40 jams and recorded dimensions of 80 key pieces. We use "key" to refer to pieces that appear to have initiated jams and "racked" to refer to all other pieces in a jam. We estimated the number of pieces in jams by first measuring the percent void space in four jams along planar transects orthogonal to racked-piece orientation. We then used a distribution of calculated piece volumes from our wood dimension measurements (see below) to estimate pieces per unit space. Finally, we measured the width, length, and height of all jams and the average piece per unit space to estimate the number of pieces in each jam.

To characterize the size, species, and age of wood in the Nisqually River, we also recorded characteristics of 173 wood pieces in three jams and on one bar. Jams were sampled along transects at a right angle to racked pieces, and the bar sample included all pieces. For both, we measured piece length and diameter, maximum and minimum rootball dimensions, and visually identified species. In these four samples, we also assigned pieces to one of three simplified decay classes: "recent" wood had most of its bark and had some limbs attached; "old" wood had no bark or limbs; and "intermediate" pieces were intermediate in characteristics.

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We conducted a similar inventory on the Snohomish River during seasonal low flow in August–October 1998 to document location, spacing, and size distribution of wood. We made this inventory in locations selected visually to represent upstream, middle, and downstream parts of the Snohomish River (river kilometres 32.2–33.9, 27.1–28.7, and 17, respectively). We measured or visually estimated diameter and length of 456 pieces and visually assigned each a decay class and species. For each piece, we noted the location as "on bar", "on bank", "snag" (a portion of the piece was emerging from the low-flow water surface), "submerged" (entirely beneath the low-flow water surface), or "embedded in bar".

We made an additional inventory in both the Snohomish and Stillaguamish rivers to estimate wood abundance. In this inventory, we counted 436 pieces in six 1-km-long reaches of the Stillaguamish River, separated by 3-km-long intervals. We also noted the channel location and depth of wood to allow us to compare current low-flow channel wood abundance to wood abundance inferred from late 1800s and early 1900s snagging documented in annual reports of the Corps of Engineers (U.S. War Department 1880–1910). Similar to the first inventory, we counted pieces according to their location on a bar, on a bank, or as a snag, or if submerged, we subjectively determined whether wood was in the thalweg of the river (in the main area of flow) or in the low-flow margins. We also noted the depth (0–0.6 m below the surface, 0.6–1.2 m below the surface, or >1.2 m below the surface). We counted 1245 pieces in the Snohomish River, counting wood in the thalweg for the entire river and the entire channel (i.e., in the low-flow margins, banks, and bars) in 10.9 km of representative locations. In the lower, tidally influenced portions of both rivers, we confined our work to minus tides to enhance our view of submerged wood. However, water clarity and visibility of wood in the deeper, tidal area of both rivers varied with turbidity and weather.

Pools

In each river, we measured pool depths using a hand-held sonar device while navigating in a canoe, rubber raft, or jet boat. We measured pool lengths and widths with a laser range finder or made visual estimates. We confirmed pool depth measurements at most jams using a stadia rod while standing at the jam edge. For each pool, we noted the dominant pool-forming influence. These include the following: free formed; wood jam (and jam type); individual wood pieces (including whether or not a rootball was attached); and valley wall or boulder forced.

Riparian forests

We documented riparian forests along the three rivers in 1866–1873, which was before widespread riparian logging, using field notes of the General Land Office cadastral surveys, which include the size and species of trees that served as witness trees at survey control points in valley-bottom forests. Surveyors were instructed to identify witness trees, four trees nearest to the survey point in each of four quadrants at section corners and two trees at midpoints between corners and at banks of navigable streams (White 1991). Field surveys of the Nisqually River riparian forest indicate that these data underrepresent smaller-diameter species such as vine maple (*Acer circinatum*) but adequately characterize the basal area (Collins and Montgomery 2002). In Indian reservations such as the Nisqually study area, survey control points were established at every quarter mile including section interiors. For clarity, we refer to these points in the immediate streamside area as "streamside" and to the regularly spaced points at section corners and fractional corners as "valley bottom".

Results

Quantity and location of wood

Wood is considerably more abundant in the Nisqually River compared with the other two rivers. The Nisqually's abundance of wood in jams accounts for most of the difference; about 90% of pieces in the Nisqually River are in jams (Fig. 2), followed by bars and banks, for a minimum total of 1400 pieces·km^{-1} or 135 pieces·CW^{-1} (channel width), including jams. Excluding jams, there were 170 pieces·km^{-1} or 17 pieces·CW^{-1}. In contrast, neither the Snohomish nor Stillaguamish rivers had significant amounts of wood in jams (Fig. 2). In both, most wood was scattered on banks or on the bed at the margins of the low-flow channel. The total abundance in both was much lower than the Nisqually: 116 pieces·km^{-1} (18 pieces·CW^{-1}) in the Snohomish and 52 pieces·km^{-1} (5.4 pieces·CW^{-1}) in the Stillaguamish. Because most wood is in jams, wood distribution in the Nisqually River is highly "bunched" longitudinally. By contrast, in the Snohomish River, wood is relatively evenly distributed. (As indicated above, our point survey of abundance in the Stillaguamish did not permit assessment of clumping.) The spatial density of wood increases in the upper 10 river kilometres of the tidally influenced reach, with the greatest amount of wood along the thalweg being at the head of tidal influence. The highest density of wood on the banks and especially the low-flow margin was in the uppermost kilometre, below where the steeper Skykomish enters the Snohomish and where, unlike downstream, banks are not armored.

Age and species of wood

Recent pieces accounted for roughly one-half (48%) of the wood pieces measured in three jams and one bar in the Nisqually (Table 1). In contrast, in the Snohomish River, old pieces accounted for 59, 41, and 96% of wood for which age-class could be determined in three sample areas, from upstream to downstream, respectively (Table 1). (A portion (28, 27, and 0%) could not be assigned to any age-class. This was typically because wood could often not be clearly viewed beneath the water surface, and so it is likely that most of these pieces not assigned to an age-class were also old because most identifiable submerged pieces were old.) Recent wood in the upstream, middle, and downstream samples accounted for only 16, 18, and 2%, respectively, of pieces that could be assigned to a decay class. Overall, wood was old, with most recent and intermediate pieces on banks; nearly four-fifths of pieces on banks were either "young" (33%) or intermediate (46%). There were also a number of recent snags in the middle and upper river samples, where eroding banks are a local source. In contrast, pieces submerged on the bed were nearly entirely old (89% of identifiable pieces).

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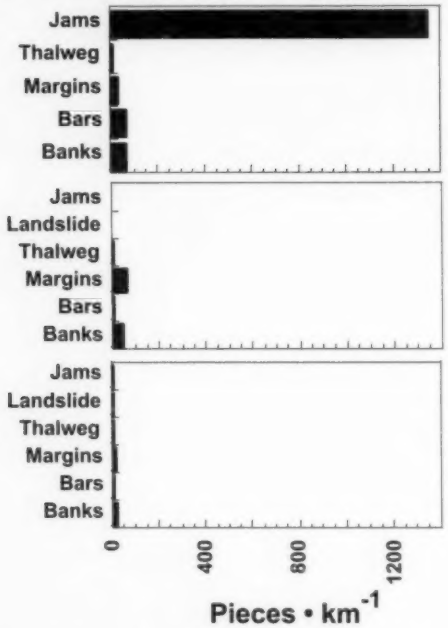
Fig. 2. Distribution of wood pieces in the sampled reach of the Nisqually River (upper panel), the Snohomish River (middle panel), and the Stillaguamish River (lower panel) study reaches.



In the Nisqually River, just over half of the wood that could be identified was conifer, distributed throughout age-classes (Table 1). In the Snohomish River, conifers (primarily cedar) dominated the population of older pieces (Table 1), whereas deciduous species were the most common recent and intermediate species.

Size and shape of wood

In the Nisqually River, recently recruited pieces are greater in diameter and length than older pieces (Fig. 3*a*), whereas in the Snohomish River, wood was dominated by smaller, older pieces (Fig. 3*b*). Wood on banks was smallest in diameter, averaging 0.33 m. Wood averaged between 10 and 12 m in length, except on bars, where wood was slightly longer, averaging 14 m. Cedar pieces were more than twice as large in diameter (mean ± standard deviation (SD) of diameter = 0.67 ± 0.29 m; unless otherwise indicated, subsequent figures also refer to mean ± SD) as alder or unidentified deciduous pieces (0.30 ± 0.06 m and 0.22 ± 0.07, respectively). Cedar was roughly half as long (6.8 ± 4.3 m) as alder or unidentified deciduous pieces (11.9 ± 4.8 m and 12.3 ± 8.6 m, respectively). Cottonwood pieces were the largest overall, with the greatest length (12.3 ± 8.6 m) and the second greatest diameter (0.35 ± 0.15 m).

Most pieces in the Nisqually River (68%) had rootballs (Fig. 3*c*). By contrast, relatively few (22%) had rootballs in the Snohomish (Fig. 3*d*); more than twice as many recent pieces as old pieces had rootballs (36 and 15%, respectively). In addition, rootballs were generally small in the Snohomish; cross-sectional area (measured as the product of the maximum and minimum rootball dimensions) averaged 2.7 ± 2.4 m^2 compared with 9.5 ± 7.2 m^2 for racked pieces in the Nisqually River. The rootballs of recent pieces in the Snohomish were slightly larger (3.3 ± 2.9 m^2) compared to those of old pieces (2.7 ± 2.2 m^2).

Most jams in the Nisqually had identifiable key pieces. Compared with racked pieces, they were longer (mean ± SD of length = 24.3 ± 8.6 m vs. 17.2 ± 9.6 m), larger in diameter (mean diameter 0.98 ± 0.34 m vs. 0.64 ± 0.36 m), and more likely to have rootballs (97% vs. 54%). Rootballs were also larger on key pieces than on racked pieces (13.1 ± 6.9 m^2 vs. 9.5 ± 7.2 m^2). Key pieces in the Nisqually ranged from 0.2 to 0.9 times the bankfull depth of 2.6 m (Fig. 3*d*). By contrast, in the Snohomish River, there were no pieces with a diameter larger than 1 m, and none of these had a rootball (Fig. 3*d*).

Historical accounts and archival data on the historical condition of Puget Lowland rivers

How representative is the Nisqually River of the mid-19th century condition of Puget Lowland rivers? Early accounts document that rivers transported vast amounts of wood. For example, in a report on the White River (Fig. 1), the Army Corps' Major Hiram Chittenden wrote

> ...the channels are strewn with immense trunks, often two hundred feet long, with roots, tops, and all ...[forming] jams, which frequently block the channels altogether. This drift constitutes the gravest feature of the flood problem, for the supply is practically unlimited, and the quantity carried by a great flood is such that very little can be done with it at the time by human agency. Levees or other protection works are of little avail in the presence of these drift jams, and it seems like an almost useless expense to built such works so long as they are menaced by so great a certainty of being destroyed or otherwise rendered useless. [Chittenden 1907]

In addition to such anecdotal accounts, archival sources also provide descriptions and quantitative information useful for characterizing the types, locations, amount, and size of wood in rivers draining to Puget Sound.

Types and locations of wood accumulations from historical archives

Raft jams

Maps and reports show that wood accumulations took various forms. "Raft" jams that formed the largest accumulations, as much as 2 km in length, were documented in the Nooksack, Skagit, and Stillaguamish rivers. These jams filled rivers from the bed to above the bank and spanned from bank to bank. A jam on the Skagit River at the town of Mount Vernon was described as 9 m deep, consisting of "from five to eight tiers of logs, which generally ranged from three to eight feet [0.9–2.4 m] in diameter" and existed for at least a century, as its surface supported live trees 0.6–1.2 m in diameter (Interstate Publishing Company 1906). A settler similarly describes "large trees growing in the midst of" (Judson 1984) a 1.2-km-long channel-spanning jam at river kilometre 8 in the Nooksack River (Fig. 1). Settlers and the Army Corps cleared mainstem-plugging jams between the late 1870s and late 1880s (U.S. War Department 1881, 1889, 1930). Individual jams of this size could have stored several hundred thousand pieces of wood.

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Table 1. Age-class and type of wood in sampled reaches of the Snohomish (SN) and Nisqually rivers.

	Age-class of wood piece														
	Unknown			Old			Int.			Recent			Total		
Location	U	C	D	U	C	D	U	C	D	U	C	D	U	C	D
SN km 32–34	21	0	5	44	55	15	10	2	40	0	5	27	75	62	87
SN km 27–29	10	4	4	9	17	3	8	4	21	3	1	11	30	26	39
SN km 17	0	0	0	91	27	1	0	0	5	0	0	2	91	27	8
Nisqually	2	0	0	25	10	6	2	12	32	2	57	25	31	79	63

Note: Int., intermediate; U, unknown; C, conifer; D, deciduous.

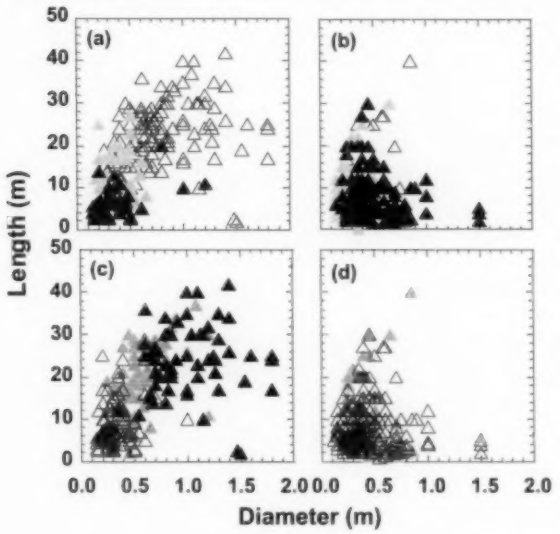

Fig. 3. Wood length, diameter, and age-class in (*a*) Nisqually River and (*b*) Snohomish River. Solid symbols, "older" pieces; shaded symbol, "intermediate" pieces; open symbols, "recent" pieces. Wood length, diameter, and presence or absence of rootballs in (*c*) Nisqually River and (*d*) Snohomish River. Open symbols, "racked" pieces without rootball; shaded symbols, "racked" pieces with rootball; solid symbols, "key" pieces with rootball.

Distributary-plugging drift jams

Extensive drift jams formed in tidal distributary channels. For example, such jams were pervasive in the Skagit River: "Only one small channel can now be navigated by steamers, the others being stopped with drift…the largest is filled from bank to bank for 3 miles" (Nesbit 1885). The regions' tidelands were reportedly characterized by "hundreds of miles" of old channels that were choked with wood, filled with sediment, and abandoned (Nesbit 1885).

These channel-plugging jams were likely important in the evolution of estuarine sloughs and marshes. Such jams are also documented in the Stillaguamish, Snohomish, and Nooksack deltas; in some cases, logging activities promoted their formation (U.S. War Department 1889–1904).

Non-channel-spanning jams and snags

Although historical maps show non-channel-spanning jams and snags, these features were not mapped systematically. However, the Army Corps of Engineers, from 1881 to the present, systematically documented their efforts to remove channel wood, or snags, to improve navigation in a federal program, concentrating on mid-channel snags. According to the Army's annual reports, snags were less abundant in the Snohomish compared with the adjacent Skagit River (U.S. War Department 1875). Settlers and surveyors indicate that snags and jams were also abundant in the Stillaguamish (General Land Office 1875; U.S. War Department 1881; Eide 1996). On an examination made in August 1879, the Army's Robert A. Habersham reported

> ….From the head of tide-water to the forks, 17 miles, the current is rapid, and the channel, which is from 125 to 200 feet wide, much obstructed by snags and trees embedded in the bottom, and at six points completely closed by rafts, which have diverted the current so as to cut out minor channels, forming small islands….The snags are numerous and large, and so deeply imbedded in the bottom that a steam snag-boat would be required for five or six months to open a channel 100 feet wide… [U.S. War Department 1881]

Quantity of wood inferred from historical records

The Army's de-snagging records can provide an indication of historical wood abundance (e.g., Sedell and Frogatt 1984). In the Puget Sound area, nearly all snagging occurred after raft jams and much of the riparian forest had already been cleared, so the number of snags recorded would be significantly less than that which would have been present under pristine conditions. Nonetheless, from 1880 to 1980, 150 000 snags were removed from five rivers, including the Stillaguamish and Snohomish, more than half from the Skagit (Fig. 4). The diminishing rate of snag removal indicates in part the decline in recruitment of large wood that would be large enough to lodge in the riverbed and remain stable. This in turn presumably reflects effects of riparian logging, especially of the largest trees, levees, and bank protection.

Earliest snagging records provide a rough, minimum estimate of historical wood recruitment. If it is assumed that snagging removed all large wood, then the amount removed in subsequent years would reflect the amount recruited to the river in the intervening winter. The inferred recruitment is likely a minimum estimate of pre-European-settlement recruitment because streamside logging had already diminished riparian recruitment. In the Snohomish River (which had fewer snags than the adjoining Stillaguamish and Skagit), initial removal (1881–1909) averaged annually 15 snags and leaning trees per river kilometre. This rate is roughly equal to the number of pieces (21 pieces per river kilometre) found in the Snohomish River in 1998 in the low-flow middle channel within 1.2 m of the low-flow surface

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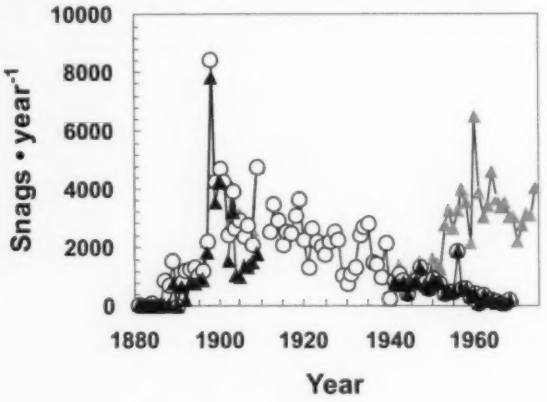
Fig. 4. Snags removed from Puget Sound rivers, 1881–1970. Open circles, all Puget Sound rivers; solid triangles, Skagit River only; shaded triangles, all Puget Sound rivers and harbors.



(Table 2), which we assume are the pieces a snagboat operator is most likely to have removed. About four pieces per river kilometre of these were recent. If we assume that recent wood encompasses pieces at most 10–20 years old, then this represents 0.2–0.4 pieces per river kilometre per year, or two orders of magnitude less than possible snag recruitment rates suggested by data from 1881–1909. In the Stillaguamish, our sampled reaches were upstream of the historically snagged reach, which was primarily the lowest few kilometres.

Size and species of wood from historical records

We know of two quantitative indicators of the size of historic wood in Puget Lowland rivers. The snagboat captain's records, which include the largest diameter of snags and trees annually removed, indicate that very large pieces were represented in the wood load, the annual maximum diameter between 1889 and 1909 ranged from 3.6 to 5.3 m (U.S. War Department 1889–1909). These very large diameters are confirmed by engineers' observations (e.g., U.S. War Department 1895).

Witness tree records in field notes of the federal General Land Office cadastral surveys include tree size and species. These are a second, more extensive data source, indicating the diameter of trees potentially recruited to the river. These trees include a significant number with diameters large enough to form key pieces in jams. For example, on the floodplains of the Nisqually, Stillaguamish, and Snohomish rivers, the largest 40, 20, and 30%, respectively, of witness trees were greater than 0.5 m in diameter, and 15, 7, and 7%, respectively, were larger than 1.0 m in diameter (Fig. 5). Trees were smaller in the immediate streamside (riparian) areas (Fig. 5). Comparing these tree size distributions with the minimum diameter of wood that form key pieces in the Nisqually, about 40% of witness trees on the Nisqually floodplain and 30% of streamside witness trees were large enough to potentially form key pieces (Fig. 3*c*).

Historical changes in geomorphic- and habitat-forming processes

To explore how historical changes in the type, size, and frequency of wood accumulations may have influenced river processes and morphology, we evaluate aspects of the riverine system at three scales: pools; channel pattern; and wood, sediment, and water routing.

Pools

We measured 85 pools in the Nisqually River study reach, a pool spacing equivalent to 1.4 CW·pool^{-1}. Roughly one-third were formed without influence of wood, 31% were formed by flow convergence at the outside of bends or at flow confluences, and 8% were formed where the river flowed against a valley-wall bluff (Fig. 6*a*). Individual wood pieces formed 18% of pools, and multiple wood pieces augmenting free-formed pools also formed 18%. Jams created the remaining one-fourth (26%). In total, wood was the dominant factor forming 61% of pools. This is similar to the finding of Abbe and Montgomery (1996; Fig. 3) in a 25-km-long reach of the Queets River where wood formed 70% of observed pools. Jam-formed pools in the Nisqually River were on average three times deeper than free-formed pools and two times deeper than pools formed by individual pieces having attached rootballs, augmented by wood, or formed by banks (Fig. 6*a*).

In the Stillaguamish River, we measured 84 pools in three sample reaches (Fig. 6*b*). Pool spacing ranged between 3.0 and 5.0 CW·pool^{-1} in the three subreaches, or one-half to one-third as many pools as in the Nisqually River. More than half formed along riprap-armored banks. Another one-quarter (27%) were free-formed alluvial pools. Only 11% were formed by wood. The remainder (10%) formed along valley-wall banks, bedrock, or boulders. Average residual pool depth was significantly greater for pools formed by riprap vs. pools along natural banks.

In the Snohomish River, we measured 18 pools in an 8.5-km-long reach, beginning at the confluence of the Snoqualmie and Skykomish rivers (Fig. 6*c*) for a pool spacing of 3.0 CW·pool^{-1} (i.e., half the number of pools in the Nisqually River). Wood formed only one relatively shallow pool. Riprap banks were the most common pool-forming factor and also formed the deepest pools (Fig. 6*c*); one such pool was 7 m deep. Several rock outcroppings in the reach were the second most important pool-forming influence and formed the second deepest pools on average.

Channel pattern and channel–floodplain interactions

The Nisqually River has an anastomosing channel pattern, with multiple main channels and numerous perennial floodplain sloughs connecting at both ends with a main channel (fig. 6*a* in Collins and Montgomery 2001). This contrasts with the Stillaguamish River, which has a single channel, except for one remaining flow split, and no floodplain sloughs. Maps and aerial photos indicate that the Stillaguamish River formerly had a channel pattern similar to that of the Nisqually (fig. 6*b* in Collins and Montgomery 2001). This same anastomosing pattern characterizes historical map and aerial photo views of several other Puget Lowland rivers. Our field studies of the Nisqually, combined with analyses of aerial photos of the river valley from 1937 to 1999 and maps beginning with 1859, indicate that wood jams caused frequent avulsions that helped to maintain multiple channels

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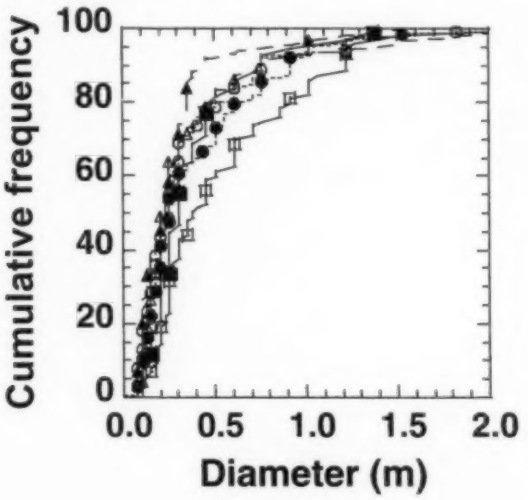
Table 2. Average number of wood pieces per river kilometre at different depths in the low-flow channel of the sampled reaches of the Snohomish and Stillaguamish rivers for use in comparison to de-snagging records (see text).

Depth category	Snohomish River, mean ± SD (pieces·km^{-1})	Stillaguamish River, mean ± SD (pieces·km^{-1})
Low-flow margin	48.5 ± 52.6 (n = 12)	22.67 ± 19.3 (n = 6)
>1.2 m	4.1 ± 8.1	0.0
0.6–1.2 m	12.1 ± 20.2	0.0
0–0.6 m	13.6 ± 13.3	3.5 ± 5.5
Snag	18.7 ± 15.6	19.2 ± 17.3
Low-flow middle	24.1 ± 50.0 (n = 21)	9.3 ± 9.8 (n = 6)
>1.2 m	3.4 ± 4.9	0.0
0.6–1.2 m	2.6 ± 4.2	0.3 ± 0.8
0–0.6 m	4.7 ± 12.1	0.5 ± 0.8
Snag	13.4 ± 35.6	8.5 ± 9.5
Total	72.6	32.0 ± 28.7

Note: Depths were visually estimated or measured. Sample size is the number of reaches. Reaches are all 1 km in the Stillaguamish; reaches of varying length in the Snohomish are normalized by reach length. SD, standard deviation.

Fig. 5. Cumulative frequency distribution of witness tree diameters from field notes of the General Land Office for the Nisqually (squares and solid lines), Stillaguamish (triangles and long-dashed lines), and Snohomish rivers (circles and short-dashed lines) in the study reaches. Solid symbols represent "streamside" trees and open symbols represent "valley bottom" trees.



and perennial floodplain sloughs (Collins and Montgomery 2002).

Water, sediment, and wood routing at the valley-bottom scale

Early descriptions indicate that raft jams diverted high flows onto floodplains. Field reports by the Army Engineers indicate that such a jam on the Skagit River at Mount Vernon (Fig. 7*a*) caused floodwaters to frequently recharge extensive (~150 m^2, U.S. War Department 1898) wetlands.

> The obstruction caused by this jam to the free flow of the flood waters prevented the low lands farther down the river being flooded, but it caused the flooding of the entire country known as the Olympia and Beaver Marsh country, to the west of the Skagit River, between the present location of the town of Avon and Padilla Bay. [U.S. War Department 1898]

Removal of the jam shifted flooding downstream and allowed settlers to drain wetlands and establish farmland (U.S. War Department 1881 (Fig. 7*b*). Enhanced routing of high flows onto the floodplain would have also routed larger amounts of fine sediment to floodplains rather than to estuarine environments. It is possible that raft jams in the region induced deposition of sediment wedges, as documented for historical Red River rafts (Triska 1984; Harvey et al. 1988).

Jams also shaped the geomorphic evolution of deltas. Removal by settlers in 1892–1893 of a jam at the head of Hat Slough, formerly a minor distributary slough on the Stillaguamish River, shifted the river's main flow into it, which has subsequently shifted the location of delta and progradation and marsh creation. Changes to a jam on the Nooksack River (Fig. 1) caused a similar course change in the mid-1800s (U.S. War Department 1893).

Discussion

Archival materials suggest that the study reach of the Nisqually River is in many respects relatively unchanged from its mid-19th century condition. The present-day distribution of tree sizes on the Nisqually floodplain closely resembles the size distribution in 1873 (Collins and Montgomery 2002), and the present-day channel pattern is similar to earlier aerial photos dating to 1937 and maps dating to 1859. It also appears reasonable to compare the Nisqually River with the historical Stillaguamish River. The mid-19th century valley-bottom forests of the Stillaguamish had tree sizes comparable to those of the Nisqually. Archival accounts indicate wood jams were abundant in the Stillaguamish River; the Stillaguamish, with a similar width as the Nisqually, formerly had an anastomosing pattern similar to that of the Nisqually.

The Nisqually is less comparable to the Snohomish. Although the Snohomish River had a riparian forest comparable with those of the other two rivers, it does not appear to

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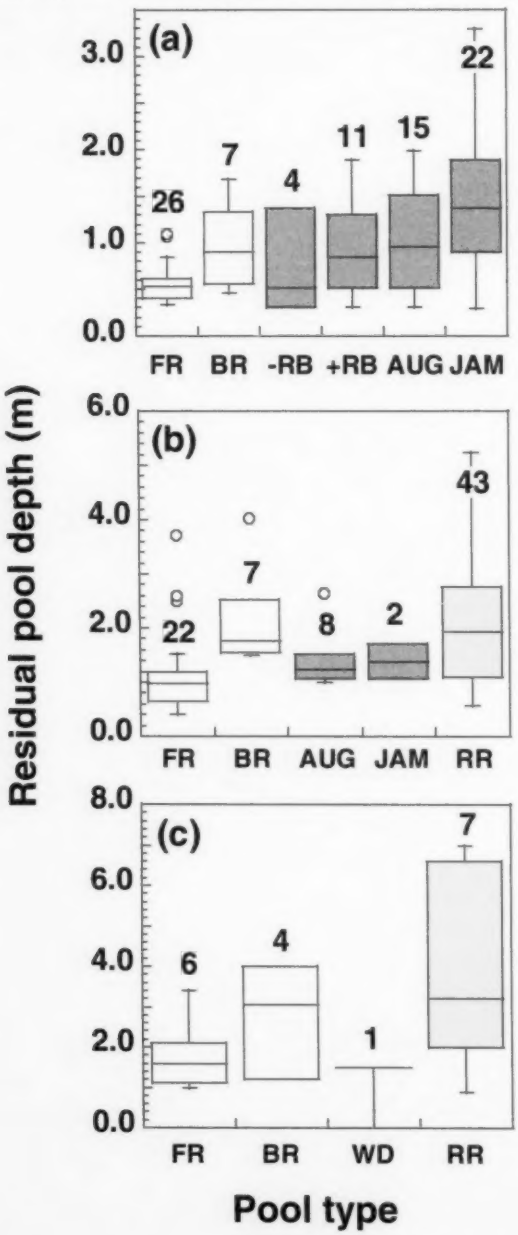
Fig. 6. Number of pools (above bars) and residual pool depth, by primary pool-forming factor, in the (*a*) Nisqually, (*b*) Stillaguamish, and (*c*) Snohomish rivers. Pool-forming factors: FR, free-formed alluvial; BR, bedrock forced; –RB, wood piece without rootball; +RB, wood piece with rootball; AUG, wood augmented; JAM, wood jam; RR, riprap armored bank; WD, wood. Numbers on plots are the sample size.



have had as much wood. This may be due in part to the larger size of the Snohomish. Perhaps more importantly, early maps show that the Snohomish did not have an anastomosing pattern but instead had a single, meandering channel with associated oxbow lakes and extensive floodplain wetlands (Collins and Montgomery 2001). Early mapping also shows that the Snohomish is similar in this regard to other rivers in broad, low-gradient valleys created by Pleistocene subglacial runoff (e.g., the Snoqualmie, Duwamish, and lower Nooksack). The Stillaguamish and Nisqually, on the other hand, are typical of rivers in valleys formed by postglacial incision (e.g., the upper Nooksack, Skykomish, and Skagit). It is possible that the two different types of river dynamics and morphologies, created by contrasting late Quaternary histories, also had different wood loads and wood functions; in particular, the less dynamic, meandering rivers in Pleistocene glacial valleys (such as the Snohomish) had less wood. Artificial channel straightening, levees and bank hardening, and the loss of streamside forests have caused the two different river morphologies and dynamics to converge in their current condition, obscuring differences that existed before European settlement that now can only be discerned from the archival record.

Comparing the Nisqually to the other two rivers in their contemporary condition shows strong contrasts. The Nisqually River had 8 and 21 times more wood than the Snohomish and Stillaguamish, respectively. Wood jams account for most of the difference; excluding jams from the Nisqually wood count, the amount was comparable with the other two rivers. We suspect that few jams occur in the Snohomish and Stillaguamish rivers for two reasons. One is the absence of long, large-diameter pieces with rootballs, which in the Nisqually River act as key pieces that initiate and stabilize jams. These largest pieces of wood are no longer available to the Snohomish and Stillaguamish rivers, which have lacked mature riparian forests for more than a century (e.g., Plummer et al. 1902). Recent wood recruitment is dominated by smaller, more readily transportable, and less decay-resistant younger hardwoods.

Another reason for fewer wood jams in the Snohomish and Stillaguamish relative to the Nisqually is the lower recruitment rate of wood, because the leveed rivers cannot laterally erode their floodplain (which also generally lacks a riparian forest). The presence of two upstream dams on the Nisqually River makes that river's greater accumulation of wood all the more remarkable and points to the importance of local wood recruitment. In contrast, neither the Stillaguamish nor Snohomish is dammed and thus have no limit on wood transport from upstream. Finally, the Snohomish and Stillaguamish rivers probably retain less wood for less time than the Nisqually, because wood is more readily transported through a river that does not trap it in jams. Jams potentially retain wood for years to decades in the active channel and then for decades to centuries or more in the floodplain when the river migrates or avulses away from a jam.

The change in size and quantity of wood that may be recruited may partially account for the paradigm that "wood is more easily transported in large channels [of the Pacific Northwest], leading to a reduction in the amount and aggregation of the remaining pieces" (Bilby and Bisson 1998). But in the Nisqually River, the abundance of wood is much greater per channel width than predicted by extrapolating from data from smaller streams (fig. 13.2 in Bilby and Bisson 1998). Thus, to some degree the generalization that wood plays a diminishing role in channel structure as

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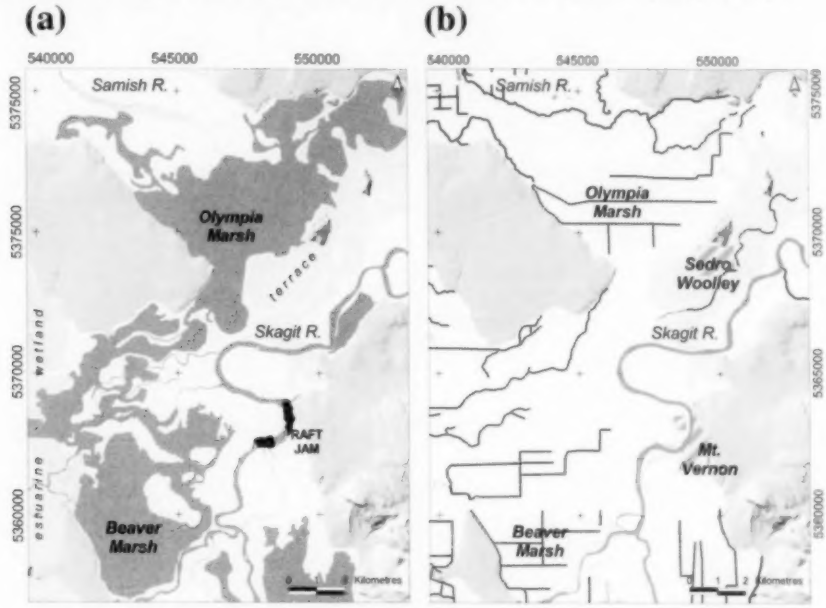
Fig. 7. (*a*) Freshwater wetlands, channels, and raft jams on the Skagit River delta in the late 1800s. Mapping is from General Land Office plat maps and field notes (1866–1873) and U.S. Army Corps of Engineers 1898 map "Index map of Skagit River, from its mouth to the town of Sedro, Washington". (*b*) The same area in 1943 from 1 : 62 500 scale topographic maps.

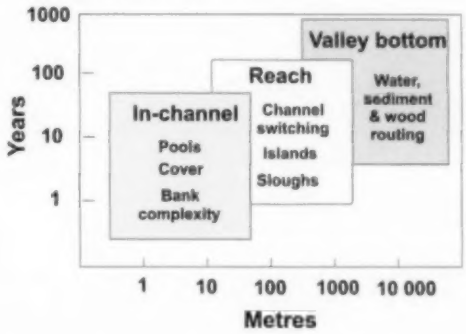


streams increase in size reflects the cumulative effect of human actions.

Large wood had a dominant influence on the geomorphology and aquatic habitat of large western Washington rivers at different temporal and spatial scales (Fig. 8). At the in-channel scale, wood formed 61% of pools in the Nisqually, comparable with the 70% reported by Abbe and Montgomery (1996) for the Queets River, but wood forms few pools in the present-day Stillaguamish and Snohomish rivers (12 and 6%, respectively). Moreover, wood-created pools, especially those created by jams, are deeper than those not created by wood. Comparing pool data from the Snohomish and Stillaguamish rivers with data from the Nisqually suggests that the transition from a freely migrating or avulsing river with mature floodplain forest to a leveed river with little riparian recruitment has reduced the number of pools by two to three times. Although artificially hardened banks appear to create deeper pools, pool depth alone is not sufficient to create high-quality habitat because wood also adds cover and complexity to pools, increasing their habitat value (e.g., Bjornn and Reiser 1991).

At the reach scale, jams in some rivers maintained multiple channels and islands and created and maintained floodplain sloughs. The historical reduction in the total amount of channel edge in the Stillaguamish River (Beechie et al. 2001) and to a lesser extent in the Snohomish River (Haas and Collins, unpublished data) reflects this simplification of channel pattern. Field studies in the Skagit River indicate that wood (particularly wood jams) significantly increases the fish habitat value of riverbanks; for example, use by subyearling chinook salmon (*Onchorhynchus tshawytscha*) was five times greater when wood was present (Beamer and Henderson 1998).

Fig. 8. The temporal and spatial scales at which wood accumulations influenced lowland Puget Sound rivers.



At the valley-bottom scale, large jams may have had a dominant influence in lower-gradient rivers on the routing of water, sediment, and wood and on floodplain-forming and floodplain-maintaining processes. Jams, especially raft jams, increased flooding and the recharge of vast floodplain wetlands in lower gradient rivers such as the Snohomish and the Skagit deltas. These wetlands would have provided extensive amounts of habitat for fish and wildlife and rearing habitat for juvenile salmonids (Groot and Margolis 1991).

The morphology and habitat of Puget Lowland rivers now differ in fundamental ways from their mid-19th century condition. Moreover, it is likely that wood abundance in rivers such as the Stillaguamish and Snohomish will continue to decline as relict wood decays and is exported; the reduced retention associated with an absence of jams has a positive feedback on wood loss. With minimal replacement from an altered riparian forest, targets or "reference conditions" for

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river and habitat restoration must take these historical changes, and likely continued change under current conditions, into account. Other temperate forest regions of the world that have experienced historical deforestation and clearing of wood from channels may have experienced changes similar in scope and magnitude to those documented here.

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Relationships between heterozygosity, allelic distance (d^2), and reproductive traits in chinook salmon, *Oncorhynchus tshawytscha*

Daniel D. Heath, Colleen A. Bryden, J. Mark Shrimpton, George K. Iwama, Joanne Kelly, and John W. Heath

Abstract: Correlations of various measures of individual genetic variation with fitness have been reported in a number of taxa; however, the genetic nature of such correlations remains uncertain. To explore this, we mated 100 male and 100 female chinook salmon (*Oncorhynchus tshawytscha*) in a one-to-one breeding design and quantified reproductive fitness and allocation (male gonadosomatic index, GSI; female fecundity; egg size; egg survival). Each fish was scored for allele size at seven microsatellite loci. We applied univariate and multivariate regression models incorporating two genetic variation statistics (microsatellite heterozygosity and squared allelic distance, d^2) with reproductive parameters. The majority of the relationships were found to be nonsignificant; however, we found significant, positive, univariate relationships for fecundity and GSI (25% of tests) and significant, multivariate relationships at individual loci for all four traits (13% of tests). One microsatellite locus, *Omy*207, appeared to be closely associated with reproductive fitness in female chinook salmon (but not male), based on the multivariate analysis. Although direct tests for overdominance versus inbreeding effects proved inconclusive, our data are consistent with the presence of both inbreeding (general) and overdominance (local) effects on reproductive traits in chinook salmon.

Résumé : Dans le passé, des corrélations ont été établies entre diverses mesures de la variation génétique individuelle et le fitness chez un bon nombre de taxons; cependant, la signification génétique de telles corrélations reste obscure. Pour étudier la question, nous avons apparié par couple 100 Saumons quinnat (*Oncorhynchus tshawytscha*) mâles et 100 femelles et quantifié leur fitness reproducteur et leur allocation reproductive (indice gonosomatique du mâle, GSI; fécondité de la femelle; taille des oeufs; survie des oeufs). Chaque poisson a été évalué quant à la taille des allèles à sept locus microsatellites. La majorité des modèles de régression à une et plusieurs variables que nous avons utilisés pour relier deux mesures de la variation génétique (hétérozygosité des microsatellites et distance allélique au carré d^2) aux paramètres reproducteurs ont généré des relations nonsignificatives; cependant, nous avons obtenu des relations significatives, positives et unidimensionnelles dans le cas de la fécondité et du GSI (25 % des épreuves) et des relations significatives multidimensionnelles pour les 4 variables à des locus individuels (13 % des épreuves). Un locus microsatellite, *Omy*207, semble fortement associé au fitness reproducteur chez les Saumons quinnat femelles (mais pas chez les mâles), d'après l'analyse multidimensionnelle. Bien que les tests directs pour distinguer les effets de la surdominance de ceux de la consanguinité n'aient pas été concluants, nos données s'accordent avec la présence à la fois d'effets généraux de la consanguinité et d'effets locaux de la surdominance sur les caractéristiques reproductives du Saumon quinnat.

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D.D. Heath,[1,2] **C.A. Bryden, J.M. Shrimpton, and J. Kelly.** Biology, College of Science and Management, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada.
G.K. Iwama. Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford St., Halifax, NS B3H 3Z1, Canada, and Faculty of Agricultural Sciences, 2357 Main Mall, Suite 249, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
J.W. Heath. Yellow Island Aquaculture Ltd., 1681 Brook Crescent, Campbell River, BC V9W 6K9, Canada.

[1]Corresponding author (e-mail: dheath@uwindsor.ca).

[2]Present address: Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada.

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Introduction

The relationship between genetic diversity and fitness has considerable theoretical and empirical support (e.g., Smouse 1986; Britten 1996; David 1998). The literature supporting a relationship between allozyme heterozygosity and measures of fitness sparked some controversy concerning the mechanism(s) of that relationship (Smouse 1986; Pogson and Zouros 1994; David 1998). One hypothesised mechanism, the "selection-based" hypothesis, was founded on the marker loci being directly involved in the fitness effect (Smouse 1986; Pogson and Zouros 1994; Pogson and Fevolden 1998). However, the use of selectively neutral genetic markers eliminates the possibility of detecting selection-based heterozygosity–fitness correlations (Pogson and Zouros 1994; Pogson and Fevolden 1998). A second postulated mechanism (the "associative overdominance" hypothesis) was based on the marker loci reflecting heterozygosity at other (functional) loci in linkage disequilibrium with the marker locus ("local" effects), or alternatively, that marker loci were in identity disequilibrium with the functional loci because of inbreeding ("general" effects) (Pogson and Zouros 1994; David 1998; Pogson and Fevolden 1998). The term "associative overdominance" is perhaps a misnomer, since it refers to effects that could be due either to overdominance or dominance at functional loci (David 1998). We will ignore the possibility of local dominance effects since they are probably rare; single-locus polymorphisms expressing dominance effects are expected to be rapidly driven to fixation (David 1998).

In theory, it is possible to distinguish local (overdominance) and general (inbreeding) effects by differences in the relative amount of explained variance from univariate and multivariate regression analyses, whereas general effects are predicted to result in a negative correlation between heterozygosity and trait variance (David 1995). Furthermore, multivariate analyses, with each locus as a separate factor, should detect local linkage disequilibrium effects at individual marker loci, which would be good candidates for quantitative trait locus (QTL) analyses.

Heterozygosity–fitness correlations are not only of theoretical interest, they are also relevant to conservation, since the management goal of maintaining genetic diversity is predicated upon the expectation that loss of genetic diversity will lead to reduced population viability (Hedrick and Miller 1992; Merola 1994). However, the link between heterozygosity and individual or population fitness is perhaps more tenuous than generally thought (Britten 1996; David 1998), making the use of indirect measures of genetic diversity, such as heterozygosity, a potentially questionable approach. Nevertheless, the use of molecular genetic markers in conservation biology is clearly valuable since such techniques have little or no impact on the study population, yet can provide a wealth of information concerning individual and population genetic history and well being (Hedrick and Miller 1992; Avise and Hamrick 1996). Additional information on the relationship between indirect measures of genetic variation and fitness is clearly needed to evaluate their use in conservation.

Here we present data on the relationship between reproductive fitness traits and estimates of genetic variation in chinook salmon (*Oncorhynchus tshawytscha*). Our goal was to test for genetic variation – fitness relationships using two statistics, microsatellite heterozygosity and mean square allelic distance, d^2, with univariate and multivariate correlation analyses and measures of reproductive fitness and fitness during early development (female relative fecundity, egg size, egg survival, and male gonadosomatic index (GSI)).

Materials and methods

The fish used in this study were reared at facilities located at Yellow Island Aquaculture Ltd. (YIAL), Quadra Island, B.C., Canada. One hundred pairs of domestic chinook salmon (aged 2, 3, and 4 years) were artificially spawned and mated in a one-to-one design in the fall of 1996. The fish were the product of four generations of captive breeding at YIAL and were descended from 10 breeding pairs of wild stock from Robertson Creek, Vancouver Island, B.C., spawned in 1985, and from an approximately equal number of fish from the same stock spawned in 1986. The farm population has since been propagated using 40–100 breeders per year, generally mated in a one-to-one breeding design. Since chinook salmon sexually mature at various ages, the farm population likely does not consist of genetically distinct cohorts.

All fish were weighed and blood was sampled for later DNA extraction and analysis before spawning. Male fish were dissected after spawning and their testes weighed. Total egg mass weight and individual egg weight (calculated from the weight of 20 eggs) were recorded for each female fish and the total number of eggs produced per fish was estimated by dividing the total egg mass by the mean individual egg weight. GSI was calculated as testes weight divided by total body weight. Relative fecundity was estimated as the calculated number of eggs per female divided by total body weight.

Eggs were dry fertilised and incubated in vertical incubation stacks (Heath Tecna Corp., Kent, Wash., and Marisource, Tacoma, Wash.). Each family was incubated in its own compartment. Water temperature during incubation was 9–10°C and flow rate was 12–15 $L \cdot min^{-1}$. Dead eggs were removed and counted at least weekly. Egg survival was calculated as the total number of eggs surviving from the "eyed stage" (developmental stage at which eye spot of developing embryo can be observed; ≈25 days post fertilization at 10°C) to hatch divided by the total number of eggs incubated (counted at eyed egg stage). We used the survival from eyed egg to hatch in preference to total egg survival because of high variability in early egg survival driven by variation in fertilization success. The mean number of eggs per family used for the survival calculation was 2990 (minimum 203, maximum 5990). Egg survival was assumed to be a female reproductive fitness trait, since maternal effects are known to be very large for both survival and growth in chinook salmon during this period (Heath et al. 1999), and thus are likely to swamp any signal from the paternal genome.

DNA extraction

DNA was extracted from blood samples using a modified proteinase K digestion method. Briefly, 3 µL of blood was digested in 200 µL proteinase K buffer (10 mM Tris (pH 8.0); 10 mM ethylenediamine tetraacetic acid (EDTA); 1% sodium dodecyl sulphate (SDS)) with 50 $\mu g \cdot mL^{-1}$

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Table 1. Primer sequences, annealing temperatures, direct count (observed) heterozygosity (H_O), expected heterozygosity (H_E), allele size range, and number of alleles observed in 200 domestic chinook salmon (100 males and 100 females).

Locus	Primer sequences (5′–3′)	Annealing temp. (°C)	H_O	H_E	Size range (bp)	Number of alleles
*Omy*325	TGTGAGACTGTCAGATTTTGC CGGAGTCCGTATCCTTCCC	56*	0.76	0.81	83–109	14
*Omy*207	ACCCTAGTCATTCAGTCAGG GATCACTGTGATAGACATCG	52	0.51	0.72§	80–108	8
*Ssa*85	AGGTGGGTCCTCCAAGCTAC ACCCGCTCCTCACTTAATC	56*	0.91	0.92	114–168	23
*Ssa*197	GGGTTGAGTAGGGAGGCTTG TGGCAGGGATTTGACATAAC	52*	0.81	0.90	146–266	34
*Ssa*293	TGGTTATTTGTTTCCAGAG ATCAGATACACAGAGACGG	52	0.62	0.69	82–92	8
*Ots*4	GACCCAGAGGACAGCACAA GGAGGACACATTTCAGCAG	56*	0.72	0.80§	137–159	12
*Sfo*8	CAACGAGCACAGAACAGG CTTCCCCTGGAGAGGAAA	52	0.76	0.90§	232–308	27

Note: PCR protocols denoted by annealing temperatures marked with an asterisk included a "touchdown" protocol of 8 cycles of –0.5°C per cycle prior to the indicated final annealing temperature (Annealing temp.).

§Significantly different from H_0 at the $P < 0.05$ level after sequential Bonferroni correction.

Table 2. Mean reproductive trait values (with standard errors, ranges, and sample sizes) for male and female chinook salmon used for genetic variation – fitness regression analysis.

Trait	Mean (±1SE)	Min. value	Max. value	*N*
GSI (%)	12.0 (±0.1)	5.2	17.8	100
Relative fecundity (no. eggs·kg^{-1})	968 (±21)	323	1687	99
Egg size (g)	0.215 (±0.006)	0.09	0.39	99
Egg survival (%)	89.1 (±1.1)	44.3	99.8	98

Note: Min., minimum; Max., maximum; GSI, gonadosomatic index; SE, standard error of the mean; *N*, number of individuals measured, or the number of families used.

proteinase K overnight at 37°C with gentle rocking. The digestion was extracted twice with phenol – chloraform – isoamyl alcohol (24:24:1) and alcohol–salt precipitated. The pellet was washed with 70% ethanol, dried, and redissolved in 100 µL water.

Microsatellite analysis

Seven dinucleotide repeat microsatellite loci were used to determine genetic variation (Table 1). The microsatellite markers used here have been described elsewhere (Olsen et al. 1996). The optimized conditions determined for chinook salmon were (*i*) a "hot-start" and a 3-min denature cycle (94°C) followed by 35 cycles of a 1-min denature step (94°C); (*ii*) a 1-min annealing step (variable temperature, see Table 1); and (*iii*) a 1.5-min extension cycle (72°C). For some primer sets, we used a touchdown protocol to increase clarity of the PCR product (see Table 1; Don et al. 1991). Reactions included 1.0 µL of the extracted DNA, 0.5 µL of each primer (100 ng·µL^{-1}), 2.5 µL of the 10× reaction buffer (Gibco-BRL), 0.5 µL dNTPs (10 mM each dNTP), 1.5 µL of 25-mM $MgCl_2$, 1.0 U *Taq* polymerase (Gibco-BRL), and ddH_2O (distilled deionized water) to make up a total 25-µL reaction volume. The forward primer for all loci was dye labeled and the resulting dye-labeled PCR fragments were run on an automated sequencer with appropriate size standards (Visible Genetics, Toronto, Ont., Canada). All microsatellite fragment sizes were determined to the nearest 0.3 bp and rounded to the nearest whole repeat number. Individual fish genotype for each of the seven loci was based on the size of the amplified DNA fragment(s), with the exception of locus *Ssa*293, which had one fixed fragment (present in 98% of fish scored) plus one or two variable fragments. The fixed fragment may represent the product of a second locus with little or no variation; genotype at locus *Ssa*293 was therefore determined using only the variable fragments. Ten of the 200 fish did not have complete microsatellite allele data: these 10 fish lacked data at one (nine fish) or two loci (one fish), despite repeated attempts to amplify the loci.

Genetic variation estimation

Two estimates of genetic variation were calculated: heterozygosity (direct count), and a genetic distance measure (d^2) based on the square of the difference in allele size in repeat numbers (Goldstein et al. 1995; Coulson et al. 1998). We logarithm transformed the mean d^2 statistic to reduce the distribution skew. Heterozygosity and log-transformed d^2 were averaged across the seven microsatellite loci for each fish, that is, heterozygosity and $\log(d^2 + 1)$ were calculated at each locus for each fish, then averaged across loci. Mean values were used for univariate trait regression analyses. Individual locus heterozygosity and $\log(d^2 + 1)$ were used as factors in the multivariate regression analyses.

Statistical analysis

Data was analysed using univariate and multivariate regression models. The univariate analysis consisted of linear regression between the various reproductive traits (dependent variable) and mean heterozygosity or mean $\log(d^2 + 1)$ (inde-

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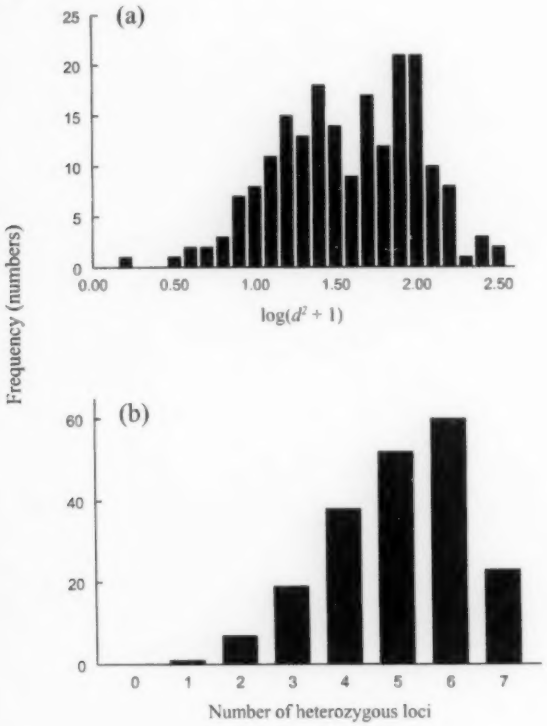
Fig. 1. Distribution of genetic variation values for the 200 chinook salmon genotyped in this study. (*a*) $\log(d^2 + 1)$ was averaged over seven microsatellite loci, whereas (*b*) heterozygosity is shown as the number of heterozygous loci counted per fish. All d^2 values were transformed ($\log(d^2 + 1)$) to reduce distributional skew.



pendent variable). Similar univariate regression analyses were performed for male and female body weight for each age class separately. The multivariate analyses were similar, except that the individual locus values for $\log(d^2 + 1)$ and heterozygosity were used as separate factors in the analysis to allow each locus to be evaluated independently. Mean egg survival was arcsine square root transformed prior to analysis, and the residuals of the egg weight – maternal body weight regression were used for the egg weight regressions. All univariate relationships were visually inspected for possible nonlinear relationships using scatterplots.

We applied tests for overdominance versus inbreeding effects as described by David (1995) to our heterozygosity – reproductive trait regressions. "Test A" is designed to compare the variance explained by univariate versus multivariate models using a likelihood ratio test; if significant, this test provides evidence for overdominance (David 1995). "Test C" is based on the expectation of a positive relationship between trait standard error and heterozygosity under the inbreeding hypothesis (David 1995).

An exact test for Hardy–Weinberg equilibrium (no allele pooling) was employed; the Monte Carlo method included a total of 2000 permutations using Tools for Population Genetic Analyses (TFPGA) software by Mark Miller (Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, U.S.A.). The resulting estimates of the probability of departure from Hardy–Weinberg equilibrium were corrected for table-wide significance using a sequential Bonferroni correction (Rice 1989).

Results

The male and female fish exhibited considerable range in reproductive allocation as measured by GSI and relative fecundity (Table 2). Absolute egg size varied from 0.0895 g to 0.3895 g and was positively correlated with female body size ($R = +0.80$; $P < 0.001$). Egg survival from the eyed-egg stage to hatch showed some variation, but most families had high survival during this period (Table 2), and there was no correlation with female body size ($R = +0.074$; $P = 0.48$). There was a strongly significant linear relationship between egg weight and egg survival ($R = +0.34$; $P < 0.0001$), but no significant relationship between the egg weight residuals and egg survival ($P = 0.88$).

The seven microsatellite loci were characterized by considerable allelic variation in allele size range, as well as in number of alleles and observed heterozygosity across all fish (Table 1). Heterozygosity and $\log(d^2 + 1)$ varied among individuals (Figs. 1*a* and 1*b*), indicating a wide range of genetic variation within our sample population, an important consideration when testing for genetic variation – fitness relationships. Three of the seven microsatellite loci were found to be significantly out of Hardy–Weinberg equilibrium (*Omy*207, *Sfo*8, and *Ots*4; see Table 1). All departures from Hardy–Weinberg equilibrium were due to an excess of homozygotes (Table 1).

Regression analyses

The univariate analyses yielded two significant regressions after Bonferroni correction: male $\log(d^2 + 1)$ with GSI, and female heterozygosity with relative fecundity (Table 3). One "marginally significant" (significant before Bonferroni correction) regression was found: female $\log(d^2 + 1)$ and relative fecundity (Table 3). The significant linear regressions of male $\log(d^2 + 1)$ with GSI (Fig. 2*a*) and female heterozygosity with relative fecundity (Fig. 2*b*) had positive slopes with R values of 0.28 and 0.25, respectively. The marginally significant relationship between female $\log(d^2 + 1)$ and relative fecundity had a positive slope and an R value of 0.20 (Table 3). Visual inspection of all univariate scatterplots showed no evidence of nonlinear relationships for any of the traits, although most univariate relationships had positive slope estimates (Table 3). No significant relationships were found between $\log(d^2 + 1)$ or heterozygosity and body weight in either the females (ages 3 or 4 years) or males (ages 2, 3, or 4 years). The age classes were analysed separately to account for body size differences in fish of different ages.

The multivariate analyses detected a limited number of significant factors (Table 4); however, all four reproductive fitness traits showed significant effects at one locus for one or both genetic variation estimators (Table 4). Most of the significant relationships are positive (Table 4). The fecundity – d^2 multivariate regression was the only one that was significant overall ($P < 0.01$; $R = 0.41$); all other multivariate regressions were nonsignificant, even before Bonferroni correc-

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Table 3. Results of the univariate regression analysis for male and female reproductive traits with mean heterozygosity and mean $\log(d^2 + 1)$.

	Heterozygosity			$\log(d^2 + 1)$		
Fitness trait	Slope	*R* value	*P*	Slope	*R* value	*P*
Male GSI	2.4 ± 1.4	0.18	0.074	**2.7 ± 0.8**	**0.28**	**0.005***
Relative fecundity	**323 ± 125**	**0.25**	**0.010***	170 ± 78	0.20	0.042
Egg size (residuals)	–0.85 ± 0.67	0.13	0.21	0.082 ± 0.49	0.02	0.87
Egg survival	0.202 ± 0.127	0.17	0.12	0.091 ± 0.078	0.12	0.24

Note: The residuals of mean egg size versus maternal body weight regression were used for the egg size regression analysis, and mean egg survival was arcsin square root transformed prior to analysis. Boldfaced values are those that remain significant (*$P < 0.05$) after sequential Bonferroni correction was applied to the heterozygosity and $\log(d^2 + 1)$ tests independently. GSI, gonadosomatic index.

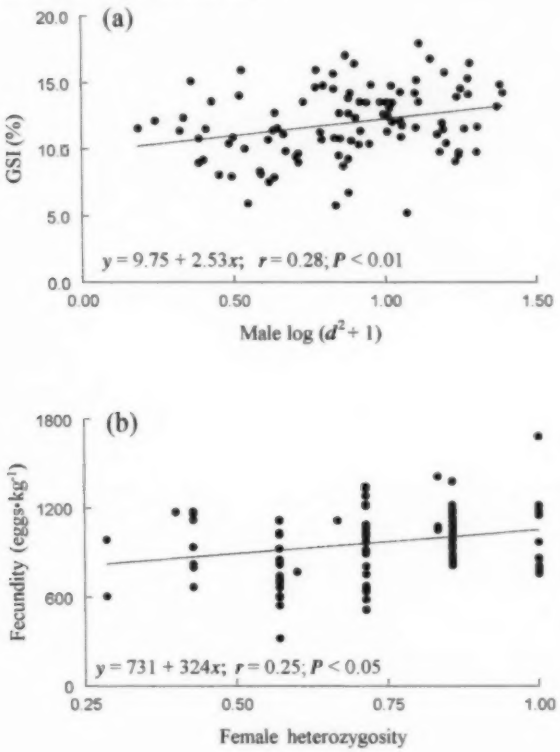

Fig. 2. Univariate linear regression of male and female reproductive traits against genetic variation. (*a*) Gonadosomatic index (GSI) versus mean $\log(d^2 + 1)$ in male fish ($N = 100$). (*b*) Relative fecundity versus mean heterozygosity in female fish ($N = 99$). Regression lines are significant at the $P < 0.05$ level before Bonferroni correction (see text).

tion. Two highly significant single-locus factors involved female $\log(d^2 + 1)$ with relative fecundity and egg survival at locus *Omy*207 (Table 4). The microsatellite locus *Omy*207 was a significant factor for all reproductive traits in female chinook salmon.

No significant difference in explained variance between the univariate and multivariate models was observed for any of the reproductive traits (Test A from David (1995): relative fecundity $F_{6,92} = 0.75$; GSI $F_{6,93} = 0.85$; egg weight (residuals) $F_{6,92} = 1.14$; egg survival $F_{6,91} = 1.71$). No significant linear relationships were found between the standard errors of the reproductive traits and heterozygosity (Test C from David (1995): $P > 0.50$).

Discussion

We found significant effects of genetic variation (both heterozygosity and d^2) on four reproductive fitness related traits in a domestic population of chinook salmon. There have been other studies reporting significant heterozygosity–fitness correlations in salmonid fish using allozyme data (e.g., Danzmann et al. 1989; Liskauskas and Ferguson 1990; Thelen and Allendorf 2001). Other studies have shown no such relationship, or a breakdown of the relationship as fish aged (Beacham 1991; Ferguson 1992). However, there has been, to our knowledge, only one other study that used microsatellite markers to examine heterozygosity–fitness correlations in fish (rainbow trout, *Oncorhynchus mykiss*), which reported no significant relationships (Thelen and Allendorf 2001). Other researchers, working with mammalian species, have reported significant correlations between genetic variation and reproductive fitness parameters (Coulson et al. 1998; Coltman et al. 1998; Slate et al. 2000). The reported significant relationships between genetic variation and reproductive life history traits may reflect the sensitivity of such traits to inbreeding effects (DeRose and Roff 1999). Our significant univariate regressions explained between 6% and 8% of the observed variance in the reproductive traits. Such R^2 values are typical of significant heterozygosity–fitness correlations (Britten 1996), although Coltman et al. (1998) reported a higher value for microsatellite d^2 and seal pup birth weight ($R^2 = 0.25$). The one study that showed significant nonmammalian microsatellite-based genetic variation – fitness correlations involved growth rates in domestic shrimp (Bierne et al. 2000). As more studies describing genetic variation – fitness correlations based on microsatellite (or other noncoding) markers in various taxa are published, we will be able to critically assess the generality of such relationships.

Chinook salmon are semelparous, and thus relationships between genetic variation and reproductive fitness are of particular evolutionary and conservation interest. Given a semelparous reproductive life history, even small changes in reproductive success could have significant implications for the evolution of life history strategies and population viability. Furthermore, a positive relationship between reproductive investment and genetic variation provides a framework for the evolution of mating barriers against inbreeding in salmon, since we show that reduced genetic variation is

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Table 4. Significance of multivariate regression analyses for male and female reproductive traits with heterozygosity and $\log(d^2 + 1)$ at seven microsatellite loci.

	Microsatellite locus						
Correlation	*Omy*207	*Ssa*293	*Sfo*8	*Ssa*85	*Omy*325	*Ots*4	*Ssa*197
Male GSI vs.							
Heterozygosity	$0.44^{(+)}$	$0.68^{(-)}$	$0.23^{(+)}$	$0.20^{(+)}$	$0.44^{(+)}$	$0.59^{(-)}$	$0.17^{(+)}$
$\log(d^2 + 1)$	$0.95^{(+)}$	$0.59^{(-)}$	$0.14^{(+)}$	$0.86^{(+)}$	$0.42^{(+)}$	$0.60^{(+)}$	$\mathbf{0.02}^{(+)}$
Relative fecundity vs.							
Heterozygosity	$\mathbf{0.01}^{(+)}$	$0.34^{(+)}$	$0.33^{(+)}$	$0.57^{(+)}$	$0.65^{(+)}$	$0.83^{(+)}$	$0.82^{(+)}$
$\log(d^2 + 1)$	$\mathbf{0.001}^{(+)}$	$0.82^{(+)}$	$0.17^{(+)}$	$0.88^{(+)}$	$0.78^{(-)}$	$0.58^{(-)}$	$0.30^{(+)}$
Egg size (residuals) vs.							
Heterozygosity	$\mathbf{0.01}^{(-)}$	$0.74^{(+)}$	$0.57^{(+)}$	$0.47^{(+)}$	$0.35^{(-)}$	$0.79^{(+)}$	$0.79^{(-)}$
$\log(d^2 + 1)$	$0.02^{(-)}$	$0.64^{(+)}$	$0.63^{(+)}$	$0.46^{(-)}$	$0.53^{(+)}$	$0.66^{(+)}$	$0.91^{(+)}$
Egg survival vs.							
Heterozygosity	$\mathbf{0.01}^{(+)}$	$0.18^{(+)}$	$0.14^{(+)}$	$0.98^{(-)}$	$0.63^{(+)}$	$0.97^{(+)}$	$0.05^{(-)}$
$\log(d^2 + 1)$	$\mathbf{0.001}^{(+)}$	$\mathbf{0.01}^{(+)}$	$0.67^{(-)}$	$0.96^{(+)}$	$0.82^{(-)}$	$0.93^{(+)}$	$0.52^{(-)}$

Note: The residuals of mean egg size versus maternal body weight regression were used for the egg size regression analysis and mean egg survival was arcsine square root transformed prior to analysis. Boldfaced significance values are those that remain significant ($P < 0.05$) after sequential Bonferroni correction. Superscripts indicate the sign of the relationship for all factors. GSI, gonadosomatic index.

associated with a measurable reduction in reproductive fitness. We observed this relationship in a domestic population of salmon that may have experienced some inbreeding, and hence our results are probably not directly applicable to large wild populations. On the other hand, the potential for a positive relationship between genetic variation and reproductive fitness may be even higher in natural populations, since such relationships are known to strengthen under suboptimal environmental conditions (e.g., Samollow and Soulé 1983; Pogson and Fevolden 1998). The salmon farm conditions in this study are presumably close to optimal, at least with respect to sequestering resources for reproduction. This possibility is supported by the observation that inbreeding depression effects increase outside of captivity (Crnokrak and Roff 1999).

Although the univariate relationships between reproductive fitness and genetic variation were stronger for male reproductive investment (GSI) than for female reproductive investment (fecundity and egg size) or egg survival, the opposite appears to hold true for the multivariate analysis (fecundity and egg survival show the strongest relationships). In theory, general effects (caused by identity disequilibrium, or whole genome effects) should manifest themselves in both univariate and multivariate analyses since general effects should, on average, affect all loci weakly, but equally. However, local effects should manifest themselves at individual loci only, and thus be detectable using multivariate analysis (although if such an effect were very large, it could drive a significant univariate relationship). Thus, our pattern of significant relationships is consistent with general effects on male reproductive investment and local effects for female reproductive fitness traits. However, specific tests for local versus general effects (described by David 1995) were inconclusive in this study, since both Test A (overdominance with local effects) and Test C (inbreeding with general effects) were nonsignificant. This is not surprising given the relatively low power of those tests for sample sizes like those in this study (approximately 100).

Our success in detecting genetic variation – reproductive fitness relationships may be, in part, a result of inbreeding within the domestic population, since moderate inbreeding is thought to increase the likelihood of detecting heterozygosity–fitness correlations (David 1998). Evidence for inbreeding in the study population includes small breeding population size, high incidence of juvenile deformities (relative to wild juveniles; C. Bryden, unpublished data), and an excess of homozygotes observed at three of the seven microsatellite loci in this study (although null alleles may also have contributed to the excess of homozygotes: see below).

We found significant relationships between heterozygosity and $\log(d^2 + 1)$ in only a small proportion of the tests performed. Significant relationships for egg size and survival were only observed in the multivariate analysis, yet both variables are highly relevant fitness parameters. Unfortunately, negative results are difficult to interpret since they may be due to biological processes or lack of statistical power. The significant departure from Hardy–Weinberg expectation observed at 3 loci (*Omy*207, *Ots*4, and *Sfo*8) may be due to inbreeding effects, Wahlund effects, or the presence of null alleles (Brookfield 1996). We used Brookfield's (1996) calculation to estimate null allele frequency at those three loci, since the positive identification of null homozygotes is difficult. The estimated frequencies of null alleles for the three loci were *Omy*207, frequency 0.12; *Ots*4, frequency 0.04; and *Sfo*8, frequency 0.07. With the possible exception of *Omy*207, the impact of potential null alleles on our analysis is not likely to be large. Null alleles are expected to bias estimates of heterozygosity, and hence to potentially mask heterozygosity – fitness relationships, yet locus *Omy*207 consistently provided the strongest relationships with our estimates of reproductive traits. Although null alleles may contribute to our observed deficiency of heterozygotes, they do not appear to represent a problem in our analysis.

The d^2 statistic has been reported to be a better genetic variation measure (relative to heterozygosity) for detecting correlations with fitness-related traits (e.g., Coltman et al. 1998, 1999; Coulson et al. 1998). Our data shows little difference between the number of significant univariate and multivariate relationships using the d^2 statistic and with that

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obtained with heterozygosity. Although empirical evidence exists for the utility of the d^2 statistic in the analysis of genetic variation – fitness correlations, an analysis of a pedigree of wolves showed heterozygosity reflected known inbreeding and outbreeding levels better than d^2 (Hedrick et al. 2001). One possible explanation for the reported advantage of d^2 for detecting genetic variation – fitness correlations may be that d^2 is better able to detect historic inbreeding or bottleneck effects. Since d^2 reflects divergence between alleles based on stepwise mutation, recent inbreeding is not likely to be reflected in the d^2 statistic unless the mutation rate is very high (Hedrick et al. 2001).

The three female reproductive fitness traits (relative fecundity, egg size, and egg survival) exhibited significant single-locus relationships under the multivariate analysis model at the microsatellite locus *Omy*207, whereas the single male reproductive trait (GSI) had no significant effect at that locus. The probability of detecting a linkage group associated with female reproductive fitness based on seven marker loci is low (Coulson et al. 1998; Pogson and Fevolden 1998; Bierne et al. 2000). However, the female reproductive traits are determined by the cumulative action of a host of different genetic factors expressed throughout the life of the salmon, and may thus be affected by a large number of loci. Irrespective of the source of the relationship, *Omy*207 would be a good candidate for inclusion in a QTL analysis for reproductive fitness in female chinook salmon.

There are very pressing concerns about the potential for loss of genetic diversity in natural Pacific salmon populations that are in decline over much of their range (Waples 1994). Loss of genetic diversity has been documented using molecular genetic markers in a number of salmon populations; however, little is known regarding the conservation implications of such trends (Waples 1994). Although genetic variation – fitness correlations generally explain little of the observed variance (i.e., low R^2), even small changes in reproductive fitness could have disproportionately large impacts on population viability, particularly if acting over long periods of time. Thus, genetic variation – fitness correlations have profound evolutionary and conservation implications (Charlesworth and Charlesworth 1987; Mitton 1993; Slate et al. 2000).

The data presented here represent one of only a handful of examples of genetic variation – fitness correlations using noncoding genetic markers; however, the number of such significant relationships is growing. Although our dataset included over 200 sexually mature chinook salmon, it was still of inadequate size to allow unambiguous testing of the local versus general effects hypotheses for the observed relationships. Nevertheless, the differences in the nature of the relationships between genetic variation for male and female reproductive fitness traits represent an important factor in the selection regime affecting male and female reproductive parameters in highly fecund, semelparous animals such as chinook salmon.

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Positive and negative effects of allochthonous dissolved organic matter and inorganic nutrients on phytoplankton growth

Jennifer L. Klug

Abstract: Dissolved organic matter (DOM) can have both positive and negative effects on phytoplankton growth. The magnitude of these effects may vary depending on the source of DOM and the composition of the phytoplankton community. Here, I address the relative importance of the positive and negative effects of DOM extracts on phytoplankton growth. In short-term experiments with phytoplankton from West Long Lake, a small, moderately coloured lake in northern Michigan, U.S.A., the net effect of doubling ambient DOM on phytoplankton growth was positive. Increasing DOM concentrations from ~10 mg $C{\cdot}L^{-1}$ to ~20 mg $C{\cdot}L^{-1}$ had a negative effect on total phytoplankton growth by reducing irradiance and thus reducing the depth to which growth was positive. However, inorganic nutrients in the DOM extracts increased growth at each irradiance level. The positive effect on phytoplankton growth owing to the nutrients associated with DOM was greater than the negative effect caused by shading. Although the positive effects of allochthonous DOM inputs outweighed the negative effects for the nutrient-limited phytoplankton in these experiments, the net effect depends on the concentration and availability of nutrients associated with allochthonous DOM as well as the physiological status of the phytoplankton community.

Résumé : La matière organique dissoute (DOM) peut avoir des effets tant positifs que négatifs sur la croissance du phytoplancton. L'importance de ces effets peut varier selon la source de DOM et la composition de la communauté phytoplanctonique. La présente étude cherche à déterminer l'importance relative des effets positifs et négatifs d'extraits de DOM sur la croissance du phytoplancton. Dans des expériences à court terme sur le phytoplancton de West Long Lake, un petit lac aux eaux moyennement colorées du nord du Michigan, É.-U., le dédoublement de la DOM ambiante a un effet positif sur la croissance du phytoplancton. L'augmentation des concentrations de DOM de ~10 mg $C{\cdot}L^{-1}$ à ~20 mg $C{\cdot}L^{-1}$ a un effet négatif sur la croissance du phytoplancton total en réduisant l'irradiance, diminuant ainsi la profondeur jusqu'à laquelle la croissance est positive. Cependant, les nutriments inorganiques dans les extraits de DOM augmentent la croissance, à tous les niveaux d'irradiance. L'effet positif des nutriments associés à la DOM sur la croissance du phytoplancton est plus grand que l'effet négatif de l'ombrage. Bien que les effets positifs des apports allochtones de DOM dépassent les effets négatifs dans ces expériences chez du phytoplancton qui est limité par la carence de nutriments, l'effet net dépendra de la concentration et de la disponibilité des nutriments associés à la DOM allochtone, de même que de l'état physiologique de la communauté phytoplanctonique.

[Traduit par la Rédaction]

Introduction

Limnologists have long recognized the importance of coloured dissolved organic matter (DOM) in aquatic systems (Birge and Juday 1927), but the net effects of DOM on primary productivity are still debated (Carpenter et al. 1998; Nurnberg and Shaw 1998; Williamson et al. 1999). DOM is a mixture of organic compounds that give brown water lakes their characteristic colour. Common constituents of DOM include organic acids such as humic and fulvic acids, and smaller molecules like amino acids and carbohydrates (McKnight and Aiken 1998). Coloured DOM may be an important regulator of autotrophic phytoplankton growth because it alters two resources that phytoplankton depend on: light and nutrients (Jones 1992; Hessen and Tranvik 1998).

DOM absorbs light and decreases the depth to which light penetrates the water column (Kirk 1996). In some clear lakes, the light absorbing properties of DOM may benefit phytoplankton by protecting them from harmful ultraviolet radiation (Ekelund 1993). However, in most lakes, DOM is more likely to be detrimental owing to decreased light available for photosynthesis (Jones 1992; Carpenter et al. 1998). In addition, decreases in light penetration in the water column may indirectly affect phytoplankton by altering the physical structure of the lake. Small lakes with high DOM concentrations typically have thinner mixed layers than lakes with lower DOM concentrations (Fee et al. 1996). The overall effect on the light environment of the epilimnion hinges on how DOM affects the size of the photic zone relative to the size of the mixed layer. For example, Jones (1992) found that DOM did not influence the average amount of light in the epilimnion of small lakes in Finland because the photic

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J.L. Klug.[1] Department of Zoology, 430 Lincoln Drive, University of Wisconsin, Madison, WI 53706, U.S.A. (e-mail: jklug@mail.fairfield.edu).

[1]Present address: Biology Department, Fairfield University, Fairfield, CT 06430, U.S.A.

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zone and the mixed layer were both shallower in highly coloured lakes.

The effects of DOM on nutrient availability are similarly diverse. Some sources of DOM contain high levels of nitrogen and phosphorus. For example, surveys have found that total phosphorus concentration is higher in lakes with high water colour and organic carbon (Meili 1992; Nurnberg and Shaw 1998). However, the availability of the phosphorus and nitrogen associated with DOM is largely unknown and involves a number of separate processes. For example, DOM may form DOM–iron–phosphorus complexes, which prevent sedimentation of phosphorus but also reduce its availability to phytoplankton (Jones 1998). Phytoplankton may be able to access the bound phosphorus if it is released by photolysis (Francko and Heath 1979; DeHaan 1992) or by production of enzymes that mediate hydrolysis of the complexes (Francko 1986). Release of nitrogen may also be increased by photolysis (Carlsson and Graneli 1993). In addition, DOM is involved in the cycling of trace elements such as iron. Studies have shown that complexes of DOM with iron and phosphorus can either increase (Prakash et al. 1973) or decrease (Jackson and Hecky 1980) the availability of iron to phytoplankton.

Because DOM can have both positive and negative effects on phytoplankton, it is difficult to predict the overall impact that changes in DOM may have on phytoplankton growth. I conducted three mesocosm experiments to explore some of the mechanisms by which DOM affects phytoplankton. In addition to the experiments, I used a simple Monod model of phytoplankton growth to illustrate the relative importance of the different mechanisms by which DOM can affect phytoplankton. Because DOM can affect both light and nutrient availability, I independently manipulated DOM, light, and inorganic nutrients to evaluate their relative influence on phytoplankton growth. Each experiment followed a similar protocol (see below), although each experiment was designed to test different hypotheses about phytoplankton response to DOM and thus contained different treatments. I expected DOM to have a negative effect on the light environment experienced by phytoplankton. In addition, I expected DOM to either increase or decrease nutrient availability to phytoplankton depending on the nutrient-binding capacity and availability of inorganic nutrients associated with the DOM source.

Methods

Study site

Long Lake is a small seepage lake located in Gogebic County, Mich. (89°32′W, 46°13′N), at the University of Notre Dame Environmental Research Center. During the 1990s, the lake was part of a set of whole-lake experiments designed to test whether lakes with different food webs responded differently to nutrient addition. In May 1991, Long Lake was divided into three basins (east, west, and central) using plastic curtains (Christensen et al. 1996). The east and west basins were used for the experiments described here. The installation of the curtain caused DOM concentrations (measured as dissolved organic carbon, DOC) to increase in East Long Lake because of changes in basin hydrology. Following curtain installation, DOC concentration was always higher in East Long Lake than in West Long Lake (Christensen et al. 1996). At the time of the experiments reported here, extinction coefficients in East and West Long lakes were 3.1 m^{-1} and ~1.7 m^{-1}, respectively. Limnological conditions in the lake at the time of the experiments are listed in Table 1.

Experimental protocol

I used 10-L square polyethylene cubitainers (I-Chem, Newcastle, Del.) as mesocosms. Water for the mesocosms was collected from West Long Lake using a 2.5 m long, 8 cm diameter PVC (polyvinyl chloride) integrated sampler between 7:30 and 9:00 to avoid light damage to the organisms. The sampler was lowered to 2 m to collect an integrated sample from 0 to 2 m. The thermocline depth ranged from 2.5 to 3 m at the time of the experiments, thus this method integrated most of the well-mixed epilimnion and avoided collection of phytoplankton from the metalimnion (Table 1). Water was passed through an 80 mm mesh net to remove cladocerans, copepods, and most rotifers. Very large phytoplankton were also excluded, but on average comprised only 3.1% of total phytoplankton biovolume (S.R. Carpenter, Center for Limnology, 680 N. Park St., Madison, WI 53706, unpublished data). Ciliates and other heterotrophic protozoans passed through the mesh. Treatments were mixed in 100-L plastic pails and then poured into the mesocosms. Lake water was diluted 1 part treatment liquid to 8 parts lake water. Control mesocosms were diluted with deionized water. The mesocosms were incubated in situ and were sampled every 1–2 days by inverting the mesocosms three times to mix and then pouring into a sample bottle.

Sources of DOM

Two sources of DOM were used in experiments: commercial *Sphagnum* peat moss (Premier Pro-Moss Sogevex, Premier Horticulture, Dorval, Que.) and freshly collected *Sphagnum* peat moss. I used DOM extracted from commercial peat moss because each experiment was designed to test a different mechanism, and a standardized DOM source was necessary to ensure consistency among experiments. To assess the effects of a different DOM source, I compared DOM extracted from commercial peat moss with DOM extracted from fresh peat moss. Commercial peat moss was extracted with deionized water. Extract was made using 140 g commercial peat moss for every 1 L of deionized water. The peat moss – water slurry was mixed every other day and left to sit at room temperature for 10 days. Extract was then coarse-filtered through muslin bags and centrifuged for 10 min at 7000 rpm (revolutions per minute). The supernatant was filter-sterilized through either a 0.2-mm or 0.45-mm filter cartridge to remove bacteria, refrigerated, and used within two days of filtering. Fresh peat extract was collected from the bog mat surrounding Tuesday Lake, a small lake less than 5 km from Long Lake. I dug a shallow (ca. 25 cm) hole in the mat approximately 30 m from the edge of the lake and collected peat from just below the water surface. Water was squeezed from the peat by hand and was filtered following the same protocol as the commercial peat moss. I measured the DOC concentration of the extract using an OI TOC (total organic carbon) analyzer (College Station, Tex.) with persulfate oxidation, pH using an electrode presoaked in sample water, and phosphorus and nitrogen concentrations using a

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Table 1. Limnological conditions in West Long Lake at the time of the experiments.

Experiment	Initial chl *a* ($\mu g \cdot L^{-1}$)	Initial DOC ($mg \cdot L^{-1}$)	TP and TN ($\mu g \cdot L^{-1}$)	Thermocline depth (m)	Depth of 1% light level (m)	k_{dPAR} (m^{-1})	Avg. irradiance ($E \cdot m^{-2} \cdot day^{-1}$)	Avg. SI ($E \cdot m^{-2} \cdot day^{-1}$)
High vs. low light	6.3	11.5	27.2, 756.5	2.5	2.7	1.8 (West) 3.1 (East)	11.6 (West) 4.6 (East)	46.4
DOM source	8.5	9.3	20.6, 620.1	2.5	3	1.5	14.9	47.1
Light gradient	4.4	10.2	22.5, 690.2	3.0	2.9	1.6	0.56–37.7	37.7

Note: Avg. irradiance, average irradiance at incubation depth; Avg. SI, average surface irradiance. DOC (dissolved organic carbon) and TP (total phosphorus) and TN (total nitrogen) refer to the ambient concentrations in the water used for the experiments. k_{dPAR} and average irradiance at incubation depth are also given for East Long Lake because containers were incubated in East Long Lake for the low light treatment of the high vs. low light experiment.

Lachat colorimetric autoanalyzer (Milwaukee, Wis.). Total phosphorus was measured following persulfate digestion and total nitrogen was measured following Kjeldahl digestion. Phosphate, nitrate, and ammonia were measured on undigested extract. The concentrations of DOC, N, and P added with the DOM extract varied because of differences in extract chemistry (Table 2). DOM treatments lowered the pH of sample water by <0.5 pH units.

NP treatments

Fertilizer for the inorganic nutrient treatments was made from potassium nitrate (KNO_3) and sodium phosphate (Na_2HPO_4). In each NP treatment, ambient P and N concentrations were elevated by 32 $\mu g \cdot L^{-1}$ and 320 $\mu g \cdot L^{-1}$, respectively, corresponding to an N–P atomic ratio of 23:1. This ratio was chosen to maintain phosphorus limitation. Preliminary experiments in West Long Lake showed that this concentration was sufficient to saturate growth with respect to nutrients (J.L. Klug, unpublished data).

Limnological analyses

Chlorophyll *a* concentration was quantified to assess changes in total phytoplankton biomass and was determined fluorometrically. Samples (100–200 mL) were filtered onto a Whatman GF/F filter. Filters were placed in film canisters and frozen for at least 24 h. Chlorophyll *a* was extracted in methanol for 24 h and analyzed with a fluorometer before and after acidification to correct for pheopigments (Marker et al. 1980). For all experiments, chlorophyll *a* concentration was determined on whole water samples. For the high light vs. low light experiment, chlorophyll *a* concentration in size-fractionated water samples was also measured. Size fractions measured were >35 µm, 10–35 µm, and <10 µm. For the DOM source experiment, phytoplankton community composition was quantified using the Utermöhl method (Utermöhl 1958). Preserved 25-mL subsamples were gravity settled for at least 22 h and counted on an inverted microscope at 400×. At least 25 fields or 200 cells were counted per sample. Most phytoplankton were identified to genus.

Surface irradiance (I_0) was measured using a model 3010 Weathertronics Mechanical Pyranograph (NovaLynx Corp., Grass Valley, Calif.), which was located in the middle of an open field approximately 1 km from the study site. Irradiance was measured continuously throughout the summer. Irradiance at depth (I_z) was measured at least weekly using a model LI-193SA LI-COR Spherical Quantum Sensor (Lincoln, Nebr.) light meter.

Experimental design

High vs. low light experiment

An experiment was designed to test how DOM affected phytoplankton growth by changing the light environment. Three replicates of eight treatments were prepared: high light control, low light control, high light NP (added inorganic nitrogen and phosphorus), low light NP, high light DOM (added DOM extracted from commercial peat moss), low light DOM, high light NPDOM (added NP and DOM), and low light NPDOM. I used the difference in DOC concentration between the east and west basins of Long Lake (see Study site section) to manipulate the light environment. The mesocosms were small enough that the light environment within the mesocosms was determined by light in the incubation environment rather than the DOM concentration in the mesocosms. All replicates were incubated at 0.75 m below the water surface. Three mesocosms each of control, NP, DOM, and NPDOM were incubated in the high DOM basin (the low light treatment), and three mesocosms each of control, NP, DOM, and NPDOM were incubated in the lower DOM basin (the high light treatment). A total of 24 mesocosms was used for this experiment. Temperature at 0.75 m was the same in both basins (~22°C). This was the only experiment in which treatments were incubated in the high DOM basin. The experiment was conducted 1–7 July 1998. Response variables were total chlorophyll *a* concentration over time and size-fractionated chlorophyll *a* concentration at the beginning and end of the experiment.

DOM source experiment

The DOM source experiment was designed to test whether DOM from different sources had similar qualitative effects on phytoplankton growth. In this experiment there were no light treatments and I used DOM extracts from both commercial and freshly collected peat moss instead of just commercial peat moss. The NP treatment was the same as in the high vs. low light experiment. A total of 18 mesocosms was used in this experiment. The experiment was conducted 23–28 June 1999. Response variables were total chlorophyll *a* concentration over time and phytoplankton community composition at the beginning and end of the experiment.

Light gradient experiment

The light gradient experiment was designed to identify the effects of DOM on phytoplankton growth at different irradiance levels. At each of nine depths in the epilimnion (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, and 2.5 m below the surface), I incubated one control mesocosm, one added NP

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Table 2. Dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) added in each experimental treatment.

Experiment	Treatment	DOC added ($mg \cdot L^{-1}$)	TN added ($\mu g \cdot L^{-1}$)	TP added ($\mu g \cdot L^{-1}$)	C:N:P of DOM
High vs. low light	Control	0	0	0	—
	NP	0	320 (320)	32 (32)	—
	DOM dried	9.9	na	na	na
	NPDOM dried	9.9	na	na	na
DOM source	Control	0	0	0	—
	NP	0	320 (320)	32 (32)	—
	DOM dried	10.9	986.6 (391.7)	30.6 (19.4)	352:32:1
	DOM fresh	5.0	190.9 (7.1)	18.7 (14.6)	263:10:1
	NPDOM dried	10.9	1306.6 (711.7)	62.6 (51.4)	352:32:1
	NPDOM fresh	5.0	510.9 (327.1)	50.7 (46.6)	263:10:1
Light gradient	Control	0	0	0	—
	NP	0	320 (320)	32 (32)	—
	DOM dried	7.9	666 (368)	19.1 (12.5)	414:35:1

Note: Values in parentheses are the concentrations of added nutrients that were inorganic (phosphate, nitrate and (or) ammonia). Nutrient ratios are by weight. DOM, dissolved organic matter; na, data not available.

mesocosm, and one added DOM mesocosm. DOM was extracted from commercial peat moss. A total of 27 mesocosms was used in this experiment. The experiment was conducted 6–12 August 1998. The response variable was total chlorophyll *a* concentration over time.

Incubating mesocosms over a range of depths allowed me to calculate the total amount of phytoplankton growth throughout the water column (areal growth). Areal growth, G, can be calculated by integrating phytoplankton growth over depth:

$$(1) \qquad G = \int_0^{z_{eu}} g(z)\mathrm{d}z$$

where $g(z)$ is growth at depth z, and z_{eu} is the depth of the euphotic zone. Irradiance decreases with depth according to Beer's law such that

$$(2) \qquad I_z = I_0 \mathrm{e}^{-k_d z}$$

where I_z is irradiance at depth z, I_0 is the surface irradiance, and k_d is the extinction coefficient of light. The change in irradiance over depth is

$$(3) \qquad \frac{\mathrm{d}I}{\mathrm{d}z} = -k_d I_0 \mathrm{e}^{-k_d z} = -k_d I_z$$

Equation 3 can be used in eq. 1 to give areal growth as a function of irradiance instead of depth:

$$(4) \qquad G = \int_{I_0}^{I_{eu}} g(I)\frac{1}{-k_d I}\mathrm{d}I = \frac{1}{k_d}\int_{I_{eu}}^{I_0}\frac{g(I)}{I}\mathrm{d}I = \frac{1}{k_d}G_0$$

where $g(I)$ is phytoplankton growth as a function of irradiance. Equation 4 gives areal growth as a function of extinction coefficient and an aggregate growth function G_0 given by $G_0 = \int_{I_{eu}}^{I_0}\frac{g(I)}{I}\mathrm{d}I$. Representing areal growth G as a function of the extinction coefficient is useful because it allows comparison of areal growth in waters with different DOM concentrations and therefore different extinction coefficients; specifically, if

$$(5) \qquad G_1 = \frac{1}{k_{d1}}G_0 \text{ and } G_2 = \frac{1}{k_{d2}}G_0, \text{ then } \frac{G_1}{G_2} = \frac{k_{d2}}{k_{d1}}$$

Therefore, if the areal growth and extinction coefficient are known for a particular lake, eq. 5 can be used to predict the change in areal growth for a given change in extinction coefficient. This method of calculating relative areal growth is only valid if the mechanism for a change in extinction coefficient (e.g., change in DOM) does not change the aggregate growth function G_0. For example, increasing DOM changes extinction coefficients but could also change the relationship between growth and irradiance by changing the spectral quality of light.

Model exercise

To illustrate the relative importance of the different mechanisms by which DOM can affect phytoplankton, I used the standard Monod model of phytoplankton growth modified to include two limiting resources (O'Neill et al. 1989; Huisman and Weissing 1995) where the growth rate μ is a function of the maximum growth rate (μ_{max}) and the external amounts of light (I) and phosphorus (P) such that

$$\mu = \mu_{max}\frac{I}{K_I + I}\frac{P}{K_P + P}$$

K_I is the light level at which growth is half of μ_{max}, and K_P is the phosphorus concentration at which growth is half of μ_{max}. Because phytoplankton growth is a function of light and phosphorus, which may both change as DOM concentration changes, this model can be used to explore how growth rates change relative to DOM concentration and source. For this exercise, I used irradiance at 1 m to calculate growth rate. I used two physiological scenarios: one scenario in which $K_I < K_P$ and one scenario in which $K_I > K_P$. These parameter values were not chosen to represent particular species but rather to illustrate the effect of phytoplankton physiology on the shape of the curves. This exercise assumes that phytoplankton are obligate autotrophs.

Statistical analyses

For the high vs. low light and DOM source experiments, I used repeated measures analysis of variance (RM-ANOVA)

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to assess time effects, treatment effects (light, DOM, and NP effects), and time × treatment interactions on chlorophyll *a* concentration (Gurevitch and Chester 1986). I applied a logarithmic transformation to normalize the residuals and homogenize variances. The symmetry of the covariance matrix did not meet the assumptions of RM-ANOVA. However, the Huynh–Feldt (H–F) conditions were satisfied, and therefore I used H–F corrected p values (Potvin et al. 1990). If a significant ($p < 0.05$) treatment or time × treatment interaction was found and there was more than one level of the treatment (e.g., in the DOM source experiment, DOM had two levels, DOM fresh and DOM dried), I used pairwise contrasts to assess which treatments were significantly different.

Results

High vs. low light experiment

The high vs. low light experiment took advantage of different DOM concentrations in the west and east basins of Long Lake by using the difference to modulate the light environment of the incubated mesocosms. Phytoplankton biomass (estimated as chlorophyll *a* concentration) was higher in the DOM (added DOM) and NP (added nitrogen and phosphorus) treatments than in the control treatment (Fig. 1; DOM effect $F_{1,16} = 66.69$, $p = 0.0001$; NP effect $F_{1,16} = 67.54$, $p = 0.0001$). However, there was a strong NP × light and DOM × light interaction, suggesting that phytoplankton response to DOM extracts and NP depended on the light environment (Fig. 1; DOM × light interaction $F_{1,16} = 46.97$, $p = 0.0001$; NP × light interaction $F_{1,16} = 24.13$, $p = 0.0001$). Phytoplankton biomass declined slightly in all of the containers that were incubated at low light levels (Fig. 1*a*). The low light levels in this experiment were caused by high DOM in the water column, which suggests a strong negative effect of DOM on phytoplankton growth via shading. In the high light treatments, phytoplankton responded positively to NP and DOM (Fig. 1*b*), and the magnitude of the response to DOM was similar to the response to NP (Fig. 1*b*). There was a marginally significant negative DOM × NP interaction, which suggests that phytoplankton response to DOM and NP in combination was less than expected based on the response to NP and DOM alone (Fig. 1; DOM × NP interaction $F_{1,16} = 4.31$, $p = 0.054$).

The phytoplankton community was initially dominated by very small taxa. Measuring the chlorophyll *a* concentrations of size-fractionated water samples showed that the proportions of <10 µm, 10–35 µm, and >35 µm taxa were 0.84, 0.06, and 0.10, respectively. In all treatments, the proportion of very small taxa declined and the proportion of larger taxa increased by the end of the experiment such that the proportions of <10 µm, 10–35 µm, and >35 µm taxa averaged 0.53, 0.24, and 0.23, respectively. The increase in larger taxa may be due to the absence of macrozooplankton grazers. Although there was a shift in size structure of the phytoplankton, the community remained dominated by very small taxa (<10 µm).

DOM source experiment

To test whether the positive effect of the DOM extracts was consistent with DOM sources other than commercial peat moss, I compared phytoplankton response to DOM extracted from dried commercial peat moss (DOM dried) with their response to DOM extracted from freshly collected peat moss (DOM fresh). Phytoplankton biomass increased in response to both sources of DOM and to NP (Fig. 2; DOM effect $F_{2,12} = 141.6$, $p = 0.0001$; NP effect $F_{1,12} = 704.95$, $p = 0.0001$). The magnitude of the phytoplankton response to fresh peat moss was less than the response to dried peat moss (Fig. 2; pairwise contrast between dried and fresh DOM $F_{1,12} = 48.05$, $p = 0.0001$). Algal biomass was highest in the NPDOM dried and NPDOM fresh treatments. Unlike the high vs. low light experiment, there was no DOM × NP interaction (Fig. 2; DOM × NP interaction $F_{2,12} = 1.47$, $p = 0.27$).

Despite large changes in phytoplankton biomass, phytoplankton community composition was not affected by the different treatments. The phytoplankton community was initially dominated numerically by *Cryptomonas*, *Closterium*, and a small, rod-shaped cyanobacteria species. However, owing to its small size, the relative biomass of the cyanobacterium was less than 5%. In all treatments, the cyanobacteria species declined dramatically, possibly because of protozoan grazers. The large increases in chlorophyll *a* concentration in the NP and DOM treatments were due to increases in *Cryptomonas*, *Closterium*, and *Cystomonas*, a nonmotile, colonial chlorophyte.

Fig. 1. Mean and standard error of chlorophyll *a* concentration throughout the high light vs. low light experiment in (*a*) the low light and (*b*) high light treatments. Note difference in scales on the *y* axes. Solid line with open circles, control treatment; solid line with solid triangles, treatment with added dissolved organic matter (DOM) extracted from dried peat moss (DOM dried); broken line with open circles, treatment with added nitrogen and phosphorus (NP); broken line with solid triangles, treatment with added DOM dried and NP.

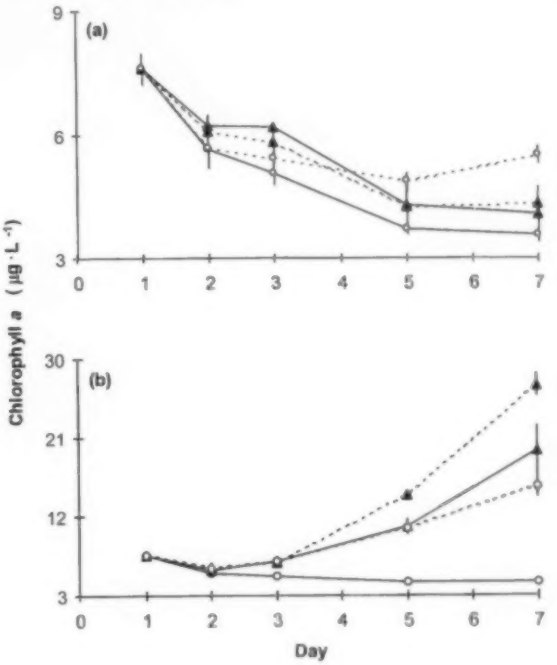

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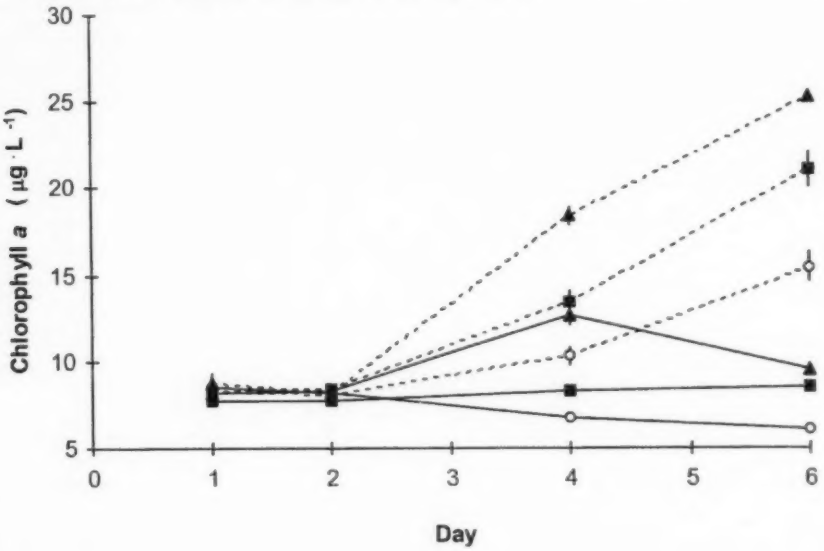
Fig. 2. Mean and standard error of chlorophyll *a* concentration throughout the DOM source experiment. Solid line with open circles, control treatment; solid line with solid triangles, treatment with added dissolved organic matter (DOM) extracted from dried peat moss (DOM dried); solid line with solid squares, treatment with added DOM extracted from fresh peat moss (DOM fresh); broken line with open circles, treatment with added nitrogen and phosphorus (NP); broken line with solid triangles, treatment with added DOM dried and NP; broken line with solid squares, treatment with added DOM fresh and NP.



Light gradient experiment

The light gradient experiment was designed to compare growth in DOM extract and NP treatments at a range of irradiance levels. Growth in the control treatment was negligible at all depths. In contrast, growth in the DOM and NP treatments was positive over most of the epilimnion (Fig. 3*a*). In addition, the growth vs. irradiance curves for DOM and NP exhibited very similar shapes, and growth in these treatments was light saturated at about 12 E·m^{-2}·day^{-1} (corresponding to depth of 0.25 m) (Fig. 3*b*). The similarity of the DOM and NP curves suggests that the amount of light removed by the DOM extract in the DOM mesocosms is small compared with the amount of light removed by the water above the mesocosms.

Areal growth for each of the treatments in the light gradient experiment was calculated by summing the change in chlorophyll *a* over all depths. In addition, I calculated areal growth in the epilimnion of West Long Lake over the 6 days of the light gradient experiment using the change in total epilimnetic chlorophyll *a* obtained from ongoing measurements in the lake (S.R. Carpenter, Center for Limnology, 680 N. Park St., Madison, WI 53706, unpublished data). I calculated areal growth in West Long Lake to compare with growth in the control treatment. Areal growth rates were highest in the NP and DOM treatments, negative in the control treatment, and very low in West Long Lake (Fig. 4).

Net effects of DOM

The high vs. low light experiment showed that DOM has a negative effect on phytoplankton growth caused by a reduction in light availability (Fig. 1). The DOM source and light gradient experiments showed that once the effect of light was removed, DOM extract had a positive effect on phytoplankton growth (Figs. 2 and 3). However, none of the experiments measured the net effect of DOM because the experimental containers were small and the elevated DOM levels in the containers did not shade the phytoplankton. Therefore, I combined the positive and negative effects of DOM observed in these experiments using the equations derived in the Methods section.

Phytoplankton growth is a function of irradiance, which means that total phytoplankton growth integrated over a given depth is a function of the extinction coefficient (eq. 4). If areal growth (G_1) is known at a particular k_d (k_{d1}) then I can calculate the expected areal growth (G_2) at k_{d2} (eq. 5; Table 3). The light gradient experiment was conducted in West Long Lake, which had an ambient DOC concentration of 10.2 mg·L^{-1} and extinction coefficient (k_{d1}) of 1.59 m^{-1} (Table 3). The observed areal growth in the DOM treatment was 1298 µg chl *a*·m^{-2}·day^{-1}. To estimate the reduction in areal growth due to reduced irradiance caused by higher DOM, I calculated the extinction coefficient (k_{d2}) using the DOC concentration in the DOM treatment (DOC = 18 mg·L^{-1}) (Table 3) and used k_{d1}, k_{d2}, and the observed growth in the DOM treatment (G_1) in eq. 5 to calculate G_2. Accounting for the estimated reduction in growth in the DOM treatment caused by reduced light leads to a net areal growth rate (G_2) of 607 µg chl *a*·m^{-2}·day^{-1} rather than the observed (G_1) 1298 µg chl *a*·m^{-2}·day^{-1} (Table 3). Nonetheless, after accounting for the negative effects of reduced light, DOM still has a large net positive effect compared with the control treatment (Fig. 4).

Model results

To explore the relative importance of positive effects of DOM owing to nutrients and negative effects owing to light

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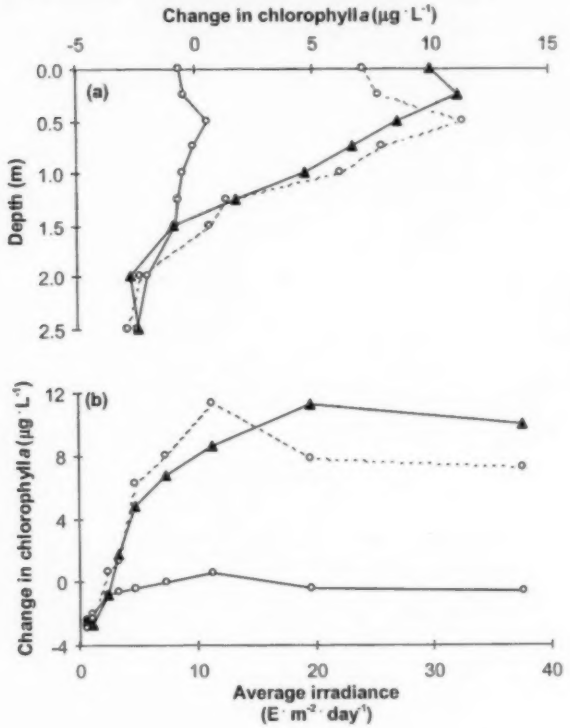
Fig. 3. (*a*) Change in chlorophyll *a* at each depth in the light gradient experiment. (*b*) Growth (change in chlorophyll *a*) versus irradiance curve for the light gradient experiment. Solid line with open circles, control treatment; solid line with solid triangles, treatment with added dissolved organic matter (DOM) extracted from dried peat moss (DOM dried); broken line with open circles, treatment with added nitrogen and phosphorus (NP).

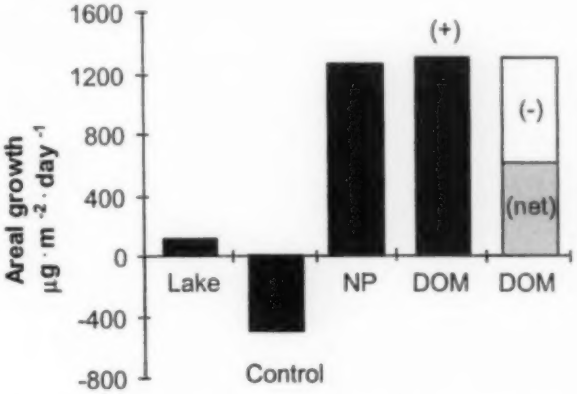


absorption, I used the Monod model of phytoplankton growth as an illustration. Model results show that phytoplankton growth rate at 1 m increases when DOM increases from low to moderate DOM concentrations and then decreases at higher DOM concentration (Fig. 5). However, the absolute effect of DOM on phytoplankton growth rate depends on the phosphorus concentration and light absorption properties of the DOM, as well as the physiological properties of the phytoplankton (Fig. 5). At low DOM, the difference in phosphorus concentration between DOM sources determines growth rate, whereas at high DOM, the difference in light absorption between DOM sources determines growth rate, suggesting that the negative effects of light become more important as DOM concentration increases. Phytoplankton that grow well at low light can better take advantage of the positive effects of DOM at higher DOM concentrations than other phytoplankton (Figs. 5*a*, 5*b*). Likewise, phytoplankton that grow well at low nutrient levels have a higher growth rate at low DOM concentrations (Figs. 5*a*, 5*b*).

Discussion

The experiments described here show a net positive effect

Fig. 4. Areal growth (μg chl $a \cdot m^{-2} \cdot day^{-1}$) for treatments in the light gradient experiment and the lake during the experiment. NP denotes the treatment with added nitrogen and phosphorus and DOM denotes the treatment with added dissolved organic matter extracted from dried peat moss. The solid DOM bar shows the positive effect of DOM extracts on phytoplankton observed during the experiment. The open bar shows the estimated negative effect of DOM extracts owing to reduction in light penetration. Reduction in growth owing to differences in light penetration was estimated using eq. 5 (Table 3). The shaded bar shows the estimated net effect (positive – negative effects) of DOM extracts on areal growth.



of increased DOM on phytoplankton growth. The net positive effect was present despite negative effects owing to shading by high levels of DOM. The relative importance of the positive versus negative effects will likely depend on a variety of factors including the source of DOM and physiological properties of the phytoplankton community present in the lake.

Negative effects of DOM on phytoplankton

Results from the high vs. low light experiment show that when phytoplankton were incubated in a low light environment they did not grow, regardless of nutrient availability. This is not a surprising result given that phytoplankton are photosynthetic organisms. In this case, the low light environment was caused by high concentrations of DOM in the lake in which the low light mesocosms were incubated, suggesting that DOM has a strong negative effect on phytoplankton owing to light absorption. When the same treatments were incubated at the same depth (0.75 m) in a lake with a lower concentration of DOM, and thus higher light, phytoplankton biomass increased in the NP and DOM treatments relative to the control treatment. The fact that growth at a given depth is lower in high DOM waters relative to lower DOM waters suggests that DOM has a negative effect on phytoplankton growth. These results are consistent with previous studies that have shown that the light absorption by DOM leads to reduction in phytoplankton growth (e.g., Jones 1992; Carpenter et al. 1998).

The effects of DOM caused by changes in light were further investigated using the results from the light gradient experiment in combination with the expected reduction in growth from increased k_d. In the DOM treatment of the light gradient experiment, increasing DOC concentration from

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Table 3. Values used to calculate reduction in growth owing to effect of reduced light.

	k_d[a] (m^{-1})	G in DOM treatment (μg chl $a \cdot m^{-2} \cdot day^{-1}$)
DOC in lake during light gradient experiment 10.2 $mg \cdot L^{-1}$	1.59	1298[b]
DOC in DOM treatment 18 $mg \cdot L^{-1}$	3.4	607[c]

Note: k_d, extinction coefficient of light; G, areal growth, DOC, dissolved organic carbon; DOM, dissolved organic matter.

[a]k_d calculated using the relationship between k_d and DOC in West Long Lake, $k_d = 0.23(DOC - 0.75)$, $n = 32$, $r^2 = 0.84$ (data are weekly summer samples from 1991 and 1992 provided by S.R. Carpenter, Center for Limnology, 680 N. Park St., Madison, WI 53706, and M.L. Pace, Institute of Ecosystem Studies, Box AB, Millbrook, NY 12545, U.S.A.).

[b]Observed growth in the DOM treatment G_1.

[c]Estimated growth in the DOM treatment if light effects are added using the relationship $G_2 = \frac{k_{d1}}{k_{d2}} G_1$ where $\frac{k_{d1}}{k_{d2}} = \frac{1.59}{3.4}$.

10.2 $mg \cdot L^{-1}$ to 18 $mg \cdot L^{-1}$ should increase k_d from 1.59 to 3.4. This increase in k_d corresponds to a predicted 53% reduction in the growth observed in the DOM treatment.

There are several other possible mechanisms by which DOM may have a negative effect on phytoplankton growth. Mechanisms involving decreases in the availability of trace minerals and possible negative effects of phenolic compounds present in the DOM are beyond the scope of this manuscript.

Positive effects of DOM on phytoplankton

The positive effects of the DOM used in these experiments were most likely due to the nitrogen and phosphorus present in the DOM extract. Arvola et al. (1996) reached similar conclusions in an experiment using DOM collected from the outflow of a bog. Similarly, Wehr et al. (1998) found that inputs of deciduous tree leaves or macrophytes significantly increased phosphorus concentrations in experimental containers. In the experiments described here, phytoplankton growth increased in the presence of added nitrate and phosphate (NP treatments), suggesting that phytoplankton in West Long Lake were nutrient limited at the time of these experiments. Phytoplankton responded to added DOM in the same way as they did to the inorganic nutrient treatments. Although the magnitude of the response to DOM was lower than NP in the DOM source experiment, the magnitude was the same in the high vs. low light experiment, and the growth vs. irradiance curves for DOM and NP in the light gradient experiment were similar.

Both of the DOM sources (fresh peat moss and dried commercial peat moss) used in the experiments were associated with appreciable amounts of inorganic and organic nitrogen and phosphorus, which may have been available to phytoplankton. The magnitude of phytoplankton response to DOM was higher for DOM derived from dried peat than for DOM derived from fresh peat. This could be due to differences in nutrient concentration and availability. DOM extracted from dried peat contained higher concentrations of dissolved nitrogen and phosphorus. In addition, very little of the nitrogen extracted from fresh peat was nitrate or ammonia; most was dissolved organic nitrogen, which is less readily used by phytoplankton (Turpin 1988).

The C–N and C–P weight ratios of DOM used in other experimental work on the effects of DOM on phytoplankton are usually higher than the extracts I used in these experiments (e.g., Tulonen et al. 1992; Vinebrooke and Leavitt 1998). There are several potential reasons for this. First, most experimental work with DOM uses different extraction methods than those used in this study (but see Arvola et al. 1996 for an exception). I used a plain water extraction, and therefore the extracts contained only readily leached compounds. In contrast, other studies filtered DOM into size fractions (e.g., Tulonen et al. 1992) or extracted with basic or acidic solutions and then dialyzed to remove loosely bound nutrients (e.g., Vinebrooke and Leavitt 1998), which may change the form of the nutrients supplied by DOM (DeHaan 1992). Chemical extraction or size fractionation of DOM is necessary when the objective is to collect large amounts of DOM or when particular fractions of DOM are needed.

Second, this study used extracts from fresh and dried *Sphagnum* peat moss, which may be richer in nutrients than other commonly used sources (e.g., soil). Peat moss is formed from undecomposed plant material, and substantial amounts of nitrogen and phosphorus are buried in peat-forming bogs (Verhoeven et al. 1990). Other studies have shown that nutrients present in peat moss may stimulate phytoplankton growth. Guildford et al. (1987) added peat moss to limnocorrals and observed short-term increases in chlorophyll *a* concentration caused by increases in soluble nitrogen and phosphorus leached from the peat moss.

I used DOM extracted from peat moss because *Sphagnum* bogs are very common in northern Wisconsin and likely contribute much of the DOM present in these lakes. Dillon and Molot (1997) found that the extent of peatlands in a catchment was correlated with the amount of DOC and total phosphorus exported to lakes in central Ontario. Gergel et al. (1999) found a similar relationship between the proportions of wetlands in a catchment and DOC concentration in northern Wisconsin lakes. The DOM produced in peat bogs and wetlands travels to lakes via surface or groundwater flow and may undergo chemical and physical changes en route. Thus, the DOM extracted from fresh and dried peat moss was likely not the same as the DOM, which enters West Long Lake but provided a consistent DOM source for use in the experiments.

When do the positive effects outweigh the negative effects?

Increasing DOM will increase the extinction coefficient of light and will decrease the amount of light available to phytoplankton at any given depth. However, if DOM contains nutrients that are available to phytoplankton, increasing DOM should increase the growth of phytoplankton at a given irradiance. Using the Monod model for growth for illustration, it is possible to explore the relative importance of the different mechanisms by which DOM can affect phytoplankton. The positive effect caused by nutrients is most important at low DOM concentrations, whereas the negative effect from reduced light becomes most important at high DOM. Although the qualitative shape of the curves is similar regardless of the physiological properties of the phytoplankton, it is likely that initial phytoplankton community composition will influence the net effect of DOM on

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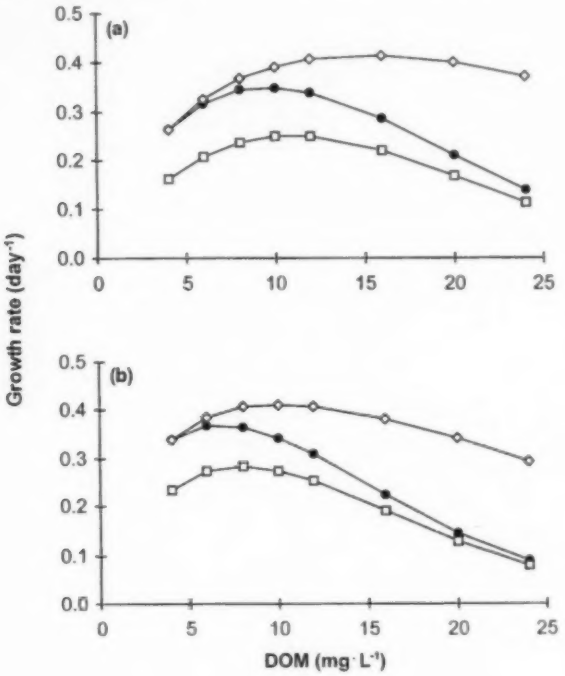
Fig. 5. Change in growth rate with increasing dissolved organic matter (DOM) concentration for (*a*) phytoplankton that grow well at low light levels (K_I = 5, K_P = 10) and (*b*) phytoplankton that grow well at low nutrient concentrations (K_I = 10, K_P = 5). Maximum growth rates (μ_{max}) are 0.8 day^{-1} in both panels. The different lines on each graph represent DOM sources with different phosphorus concentrations or different light absorption properties. The line with the solid circles represents DOM with the characteristics of DOM extracted from fresh peat moss. The line with the open diamonds represents a DOM source that is half as coloured as peat moss DOM and the line with the open squares represents a DOM source with half as much phosphorus as peat moss DOM.



phytoplankton growth. West Long Lake is a moderately coloured lake and the phytoplankton community at the time of the experiments was dominated by cryptophytes, which are common in lakes with high DOM (Jones 1998). Cryptophytes are motile and able to grow at low light levels (Klaveness 1988), which may explain their prevalence in coloured lakes. In lakes with low DOM, where phytoplankton are adapted to high light levels, the negative effects of DOM owing to reduced light may outweigh the positive effects owing to nutrients.

The simple model assumes that phytoplankton are obligate autotrophs. However, there is evidence that some phytoplankton species (including some cryptophytes) are mixotrophic (Bird and Kalff 1987; Tranvik et al. 1989). The importance of mixotrophy is debated but it has been suggested that mixotrophic species may be better adapted to high DOM lakes than are obligate autotrophs. For example, some studies have shown that known mixotrophs are more prevalent than autotrophs when organic carbon supply is high (Jansson et al. 1999; Grover 2000). It is possible that some cryptophyte species could have responded positively to DOM additions because of their mixotrophic capabilities. However, some of the noncryptophyte species (e.g., *Cystomonas starrii*) that responded strongly to additions of nutrients and DOM have no known mixotrophic capabilities.

The dominance by cryptophytes may also help explain why growth in the control treatments was lower than growth in the lake. The increased phytoplankton growth in the nutrient treatments suggests that phytoplankton in West Long Lake were nutrient limited. Therefore, the negative growth in the control treatments may be due to lower nutrient availability in the mesocosms relative to the lake. Some cryptophyte species undergo diurnal vertical migrations, which give them access to nutrients in the hypolimnion (Salonen et al. 1984). Phytoplankton within the mesocosms did not have access to that nutrient source. In addition, the absence of nutrient recycling by zooplankton and fish could lead to lower nutrient availability in the control treatments relative to the lake.

Although there were strong treatment differences in total phytoplankton biomass, phytoplankton community composition was not substantially different among treatments. These results are likely due to the short time scale of the experiments, as longer-term studies have shown that changes in DOM do affect phytoplankton community composition (Wehr et al. 1998; Klug and Cottingham 2001). The experimental manipulations presented here represent short-term pulses of DOM as might occur following storm events or spring snow melt. Previous work has shown that DOM supplied during these short-term episodes has different characteristics than DOM supplied during baseline hydrologic conditions (Ivarsson and Jansson 1994; Jansson et al. 1999)

In summary, in many cases, it is only possible to see the net effect of DOM on organisms. However, by separating the nutrient effects from the light effects, I showed both positive and negative effects of DOM on phytoplankton growth. The relative effects of light and nutrients will depend on the characteristics of the DOM as well as properties of the phytoplankton community. Experimental results showed that in moderately coloured, nutrient-limited lakes such as West Long Lake, the positive effects exerted by inorganic nutrients associated with DOM outweighed the negative effects of decreased light. Because the observed positive and negative effects of DOM were a function of the nutrient availability and colour of DOM, different sources of DOM will have different net effects on phytoplankton growth. Distinguishing the mechanisms by which DOM affects phytoplankton growth and improving techniques for characterizing the availability of nutrients associated with DOM should help predict how phytoplankton will respond to different DOM sources.

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Recruitment overfishing in a bivalve mollusc fishery: hard clams (*Mercenaria mercenaria*) in North Carolina

Charles H. Peterson

Abstract: Because of their high fecundity, marine invertebrate fisheries are rarely considered at risk to recruitment overfishing. This presumption can be criticized on population theoretic grounds and conflicts with growing evidence of recruitment limitation in a variety of marine invertebrate populations. Sampling in 11 years spanning a 24-year period from 1978 to 2001 reveals that hard clam (*Mercenaria mercenaria* (L.)) recruitment declined significantly by 65–72% within the fishing grounds of central North Carolina. This 24-year period began when high demand and prices drove increased clamming effort. Accordingly, landings grew rapidly 5-fold, a yield that was not sustained and subsequently fell by over 50% from 1983 to 2000. Fishery-independent sampling repeated identically in three representative habitats demonstrates declines of 17, 79, and 95% in hard clam density and of 24, 46, and 83% in spawning stock biomass during the 18+ years of 1980–1997. Small-scale experiments and measurements in depleted habitats show no compensatory enhancement of hard clam recruitment with local reduction in adult density. Consequently, the hard clam in North Carolina serves as perhaps the most compelling example of unsustainable fishing mortality leading to recruitment overfishing in a bivalve mollusc stock. Spawner sanctuaries could serve to restore and protect spawning stock biomass in this and other invertebrate fisheries.

Résumé : À cause de leur fécondité élevée, les invertébrés marins d'intérêt commercial sont, croit-on, rarement menacés par la surexploitation du recrutement. Une telle supposition peut être critiquée sur la base de la théorie démécologique et elle est aussi contredite par des indices de plus en plus nombreux de l'existence d'une limite au recrutement chez plusieurs populations d'invertébrés marins. Un échantillonnage de 11 années réparti sur 24 ans (1978–2001) révèle que le recrutement de la Palourde américaine (*Mercenaria mercenaria* (L.)) a baissé significativement de 65–72 % sur les territoires de pêche du centre de la Caroline du Nord. La période de 24 ans a débuté à un moment où une forte demande et des prix élevés ont entraîné un effort accru de la pêche. Il en a résulté naturellement une croissance rapide des débarquements qui ont quintuplé, mais ce rendement ne s'est pas maintenu et il a finalement chuté de plus de 50 % de 1983 à 2000. Un échantillonnage mené indépendamment de la récolte commerciale et réitéré dans trois habitats représentatifs indique des déclins de 17, 79 et 95 % des densités des palourdes et de 24, 46 et 83 % de la biomasse du stock reproducteur durant la période de 18+ années, de 1980 à 1997. Des expériences à petite échelle et des mesures dans des habitats épuisés n'indiquent aucune augmentation compensatoire du recrutement de la Palourde américaine associée à la réduction de la densité des adultes. En conséquence, les Palourdes américaines de la Caroline du Nord sont peut-être l'exemple le plus convaincant, chez un stock de mollusques bivalves, d'une mortalité due à la pêche menaçant la durabilité de la ressource et menant à la surexploitation des reproducteurs. L'établissement de sanctuaires pour les reproducteurs pourrait aider à restaurer et protéger la biomasse du stock des reproducteurs chez cette espèce et d'autres invertébrés d'intérêt commercial.

[Traduit par la Rédaction]

Introduction

Although the traditional approach of managing fisheries through use of population-based models to identify the level of harvest that produces the optimal sustainable yield has been criticized on numerous grounds (e.g., Larkin 1977), assessing the underlying relationship between adult spawning stock and recruitment still represents a fundamental imperative to sustainable fisheries management (Myers and Barrowman 1996). Classic fishery dynamics models used for management typically assumed compensatory relationships between spawning stock abundance and survival, and thereby recruitment, of their progeny. This was because of competition for food and other negatively density-dependent processes in early life history stages (Ricker 1954; Beverton and Holt 1957; Cushing 1971). Yet, recruitment overfishing can occur if adult spawning stock biomass is reduced by such intense harvest as to lower the recruitment derived from progeny of those overfished generations of adults. This outcome represents the generally most serious error that can be made in fisheries management, and, if the recruitment overfishing is severe enough, can lead to effective elimination of the commercial fishery. The review of stock–recruit relationships in finfish by Myers and Barrowman (1996)

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C.H. Peterson. University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, NC 28557, U.S.A. (e-mail: cpeters@email.unc.edu).

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clearly shows that maintaining adequate spawning stock is critical to sustainable fisheries for most exploited finfishes. There are even conceptual reasons based on trophic interactions and fertilization success that may lead to depensatory (positively density dependent) relationships between spawning stock abundance and survival of their young (Quinn et al. 1993; Pfister and Bradbury 1996; Walters and Kitchell 2001). These add further urgency to protecting spawning stock biomass in fisheries management.

Concerns about recruitment overfishing rarely enter into management plans for invertebrate fisheries, especially those for bivalve molluscs (e.g., Hancock 1973). The rationale used to support exclusion of stock–recruit relationships from management plans for bivalve and most other invertebrate fisheries is based upon their high fecundity, long larval period, and widely dispersed adult distribution (Jamieson 1993). An adult female bivalve produces millions of eggs annually, and typically reaches an early reproductive maturity with multiple years of spawning. The contrast in life history with a typical finfish, or especially with an elasmobranch that gestates its young, is dramatic. Those few bivalve mollusc fisheries in which protecting spawning stock biomass is fundamental to management are usually exceptional in some way. For example, bay scallops (*Argopecten irradians*) are annuals, so to avoid substantial depletion of the parental stock, the fishing season in North Carolina opens after spawning has been completed (Peterson 1990). In addition, because bay scallops have short larval periods and are patchily distributed geographically such that populations are isolated by water basin, spawner transplantation has been practiced to restore commercially defunct fisheries (Peterson et al. 1996). Yet management of most bivalve fisheries worldwide assumes that high fecundity will sustain historic levels of recruitment without need to protect spawning stock beyond setting minimum legal body size at harvest (Jamieson 1993).

The logic underlying a decision to manage bivalve mollusc fisheries without regard to potential stock–recruitment relationships can be challenged on grounds of theory and on the basis of empirical observations. A life history characterized by high fecundity is also characterized by high mortality. That is, production of large numbers of progeny is required merely for replacement of the adult generation. High fecundity does not translate directly into a reduced need for reproductive adults to sustain population size. Only if strong compensatory mortality processes operate on the early life history stages does high fecundity imply enhanced capacity for sustaining recruitment over wide ranges of spawning adult population size. We know very little about density-dependent processes in the early life history stages of marine organisms (Morgan 2001), but there is no compelling theoretical basis for assuming that they operate more consistently and more powerfully on highly fecund species. For most invertebrates in the soft-sediment habitats occupied by exploited bivalves, postsettlement processes are known to have important effects on recruitment and abundance (reviewed by Olafsson et al. 1994), but there is no basis to conclude that these effects regulate species abundances to fall within some narrow range independent of settlement intensity. A species with low fecundity but in which compensatory mortality processes are strong could achieve high recruitment in the face of depletion of adult spawners better than a highly fecund species with no compensatory mortality acting during prerecruit development. Other exploited marine invertebrates like abalone and sea urchins with life histories similar to bivalves, although with generally lower fecundity and shorter larval periods, exhibit evidence of recruitment limitation (Jamieson 1993).

Here, I exploit long-term data sets for hard clam (*Mercenaria mercenaria* (L.)) recruitment and for fishery-independent information on changing hard clam abundance in central North Carolina to test whether recruitment changed over a two-decade period characterized by greatly increased harvest pressure and declining clam stocks. The results imply operation of a strong and detectable relationship between spawning stock biomass and recruitment in hard clams over the range of variation exhibited in the past two decades. Changes in hard clam management in North Carolina and elsewhere throughout the species' range and for bivalve mollusc fisheries generally are important to achieve sustainability.

Materials and methods

Hard clam fishery in North Carolina

Hard clams represent an important fishery in North Carolina, with catches ranging from 577 000 to 1 702 000 pounds (1 pound (lb) = 0.453 kg) of meat over the past 21 years (1980–2000), worth from $3 774 000 to $8 388 000 U.S. at dockside (North Carolina Division of Marine Fisheries, Statistical Records, Morehead City). This fishery is very important economically, varying from between first and fourth in dollar value in the state over this period. Prior to 1977, fishing pressure on North Carolina hard clams was not intense. Hard clams represented a resource that was effectively banked by the fishing industry like living capital in clam beds until needed, thereby serving as a social and economic safety net. Because of the longevity of hard clams, beds of them persisted indefinitely until hard times in other fisheries forced fishermen to turn to clamming to sustain their incomes. As increasing prices paid for hard clams in the late 1970s drove substantial jumps in fishing effort in North Carolina, harvest increased 5-fold from 1976 to 1980 (Peterson et al. 1983). Widespread adoption of clam kicking, a mechanized form of harvest (Guthrie and Lewis 1982), enhanced effort by several fold: records available on numbers of permits support this inference. More explicit data on effort were not collected at that time in part because of an absence of appreciation for the potential need to protect spawning stock biomass to sustain the fishery. Presently in 2001, few boats are outfitted to kick clams because, despite high prices, clam stocks in the kicking grounds are depleted to a level that does not allow economically viable fishing. To date, no fishery-independent data on hard clam stocks are routinely collected in North Carolina and the landings data cannot be reliably used to infer stock size because of the absence of detailed long-term effort information and the confounding of wild stock landings with hatchery production in the legally publishable portion of the North Carolina fisheries data base.

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Hard clam recruitment

I have been involved in gathering quantitative data on annual recruitment of hard clams using identical methods over a period of 24 years (1978–2001). Annual recruitment of hard clams was estimated by twice systematically sieving the top 10 cm of surface sediments in replicate 1-m^2 enclosures by hand through 6-mm mesh (methods in Peterson et al. 1983). The assessment of recruitment was conducted in early October in each of 11 years (see Table 1 for specific schedule of replication by year) so as to provide a measure that integrates successful recruitment over the entire settlement season. This definition of recruitment follows that used by marine ecologists (e.g., Keough and Downes 1982) rather than the size of entry into the fishery because I am interested in the measure that comes closest to assessing whether larval settlement is altered by changing spawning stock biomass.

The 1-m^2 enclosures sampled for recruits were located in shallow subtidal bottom at a site in Middle Marsh, Back Sound, within the Rachel Carson NOAA National Estuarine Research Reserve (Fig. 1; site MI on map in Peterson et al. 1983). Because of the tidal current flow driven through Beaufort Inlet nearby and the proximity of southern Core Sound and the Straits connecting Back Sound with Core Sound, the waters bathing Middle Marsh contain larvae from hard clams occupying most or all of the prime commercial clamming grounds of the central coast of North Carolina (Core and Back Sounds, North and Newport Rivers). The enclosures used for this sampling were designed so as to be able to manipulate densities of adult and juvenile clams without risk of migration interfering with the density treatments. Enclosures were constructed from 4.2-m × 13-cm (length × height) strips of Vexar black plastic 6-mm mesh and folded to enclose a 1-m^2 area. They were forced 10 cm into the sediments. To anchor the enclosures, 0.6-m steel reinforcing rods were also pushed into the sediments and attached by nylon cable ties to the Vexar mesh at each corner and at halfway points of each side wall.

In the four early years of sampling these enclosures (1978–1981), adult hard clam density was varied systematically among enclosures (see Table 1 for experimental design) so as to test whether local clam density affected hard clam recruitment. Hard clams used in these density manipulation experiments were marked with color-coded dots of nontoxic Mark-Tex Corp. (Olathe, Kans.) paints on the outer shell near the umbo. Because all enclosures were cleared of hard clams by sieving before establishment of the density treatments, recruits could be identified unambiguously by appearance of unmarked small clams. We measured the length of each recruit recovered during sieving with Vernier calipers and plotted size-frequency distributions to allow us to define recruits by size at that season of the year. Two size cutoffs can be justified from examination of these size distributions, one at 15 mm and another at 26 mm in length (see size-frequency curves in Peterson et al. 1983). The majority of the recruits fall below the 15-mm cutoff, but a few faster growers or early settlers occur in the 16- to 26-mm range. In the last seven years of sampling for recruits inside these fixed enclosures (1983, 1984, 1986, 1987, 1994, 1995, and 2001), adult clam density inside the enclosures was not systematically varied.

Table 1. Schedule of October sampling to measure seasonal recruitment totals for hard clams (*Mercenaria mercenaria*) and mean densities of recruits ≤15 mm long.

Year	Adult density (m^{-2})	Replication	Mean density of recruits ≤15 mm long
1978	0	5	2.6
	2	4	1.5
	7	5	1.8
	28	5	2.0
	Pooled	19	2.0
1979	0	6	4.0
	2	5	2.4
	7	6	0.8
	28	6	0.8
	56	6	2.7
	Pooled	29	2.1
1980	0	2	1.5
	7	6	1.7
	112	3	1.3
	Pooled	11	1.6
1981	0	3	2.7
	7	6	0.5
	56	3	0.7
	112	3	0.7
	Pooled	15	1.0
1983	Variable	50	2.9
1984	Variable	42	1.2
1986	Variable	17	4.3
1987	Variable	24	0.3
1994	Variable	37	0.8
1995	Variable	31	1.1
2001	Variable	25	0.8

Note: Recruitment was assessed by sieving fixed 1-m^2 plots by hand through 6-mm mesh. Plots were located at site MI (see Fig. 1) within Middle Marsh in Back Sound, N.C. After 1981, adult densities were not experimentally fixed but varied from 0 to 12 $m.^{-2}$

Hard clam density and spawning stock

Because of the absence of any standardized stock assessment of hard clams to provide fishery-independent data on status and trends, in November 1997 I resampled three areas that we had previously sampled over 18 years before. A temporal comparison of densities in those three areas provides quantitative evidence with which to evaluate the widely held conviction that hard clam stocks in central North Carolina have been greatly depleted from the late 1970s to the late 1990s. The three sites previously sampled, then resampled, are all marked on Fig. 1: (*i*) a tributary to Johnson Creek, a muddy sand tidal creek extending from Core Sound into Core Banks in the region where intense clam kicking was conducted in the late 1970s and early 1980s; (*ii*) a shallow subtidal sand flat at Baldhill Bay; and (*iii*) a nearby shallow subtidal seagrass bed at Twelve O'Clock Shoal, both in Back Sound close to Shackleford Banks. The site boundaries were chosen to match exactly those used to delineate them at the earlier samplings. No changes had occurred in the appearance of the habitats at any site: we took care to sample the identical habitat that we had sampled previously. Johnson

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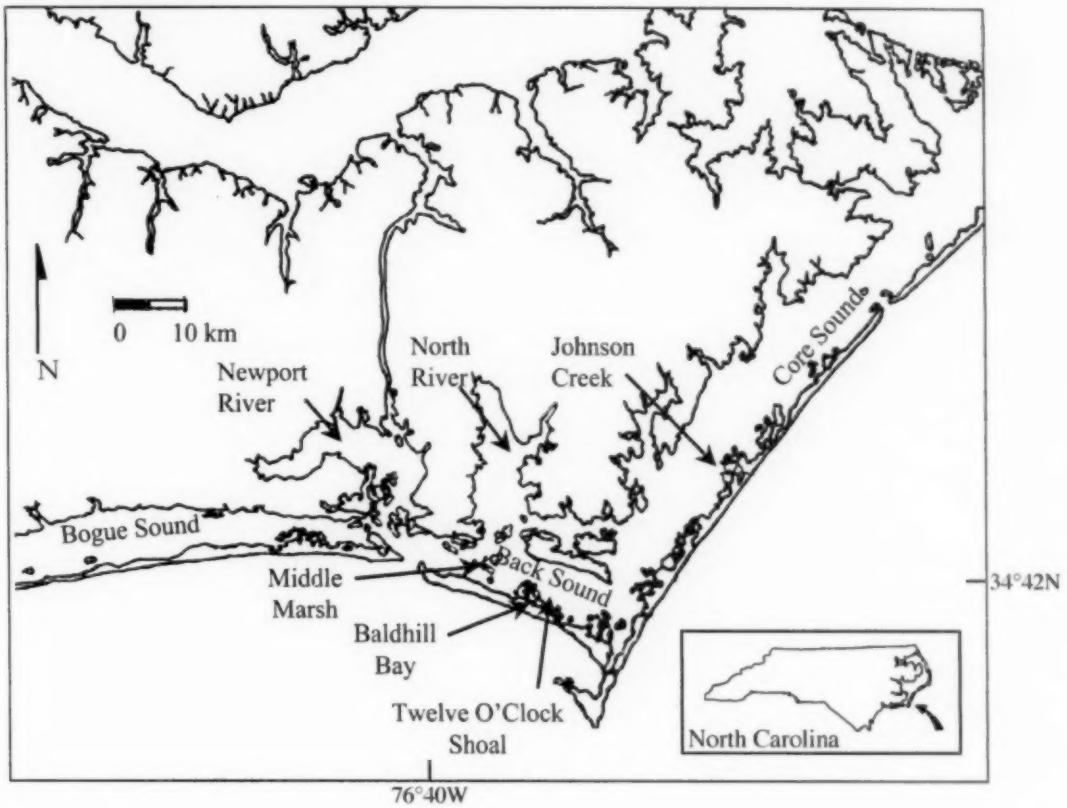
Fig. 1. Map of the central coast of North Carolina, U.S.A., showing the Middle Marsh site for experimental manipulations of hard clam (*Mercenaria mercenaria*) density and of repeated sampling of annual hard clam recruitment, as well as the three sites resampled after 18+ years, a tributary of Johnson Creek, Baldhill Bay, and Twelve O'Clock Shoal. Inset map shows the state of North Carolina, with study site location indicated.



Creek was sampied first on 15 February 1980 by excavating with a suction dredge 24 haphazardly located 0.25-m^2 sampling frames and sorting contents on a 3 mm mesh bag and by applying the hand-sieving technique through 6-mm mesh in 10 additional haphazardly located 0.25-m^2 sampling frames (see Methods in Peterson et al. 1983). Resampling was done on 25 November 1997 using the identical suction dredge methodology but with 36 replicate 0.25-m^2 samples. The sand flat and seagrass bed sites were first sampled quantitatively using this same suction dredge methodology in winter–spring 1980 from 22 February to 6 May, with 228 0.25-m^2 suction dredge samples taken at each site (Peterson et al. 1987). Resampling was conducted at both sites on 21 November 1997 using 36 0.25-m^2 suction dredge samples from the sand flat site and 21 from the seagrass bed site. Resampling was restricted to the two control matrices, avoiding the four matrices that had been subjected to experimental harvesting treatments (Peterson et al. 1987). Hard clams were counted and collected for measurement of shell length and aging (Peterson et al. 1984) by analysis of annuli (Peterson et al. 1983). Because I had previously conducted an evaluation of how gonadal mass in hard clams from this central region of North Carolina varies with shell length (Peterson 1983), I used this knowledge along with the observed size-frequency distributions to calculate estimates of the total gonadal mass of all hard clams per unit area at each site in spring 1980, and again in the year 1997. The total gonadal mass per unit area is used as an index of spawning stock biomass for each site. For these calculations, I used the length – gonadal mass regression for prespawning (May) clams, as appropriate to assess reproductive potential.

Results

In no year of the four when experiments were conducted did recruitment of hard clams vary significantly with adult density inside the 1-m^2 enclosures (Tables 1 and 2). This conclusion held true for recruits defined by either the 15- or 26-mm size limit. The p values in single factor analyses of variance (ANOVAs) conducted on the recruitment results of each year's density manipulation experiment were high in all four years (Table 2), implying low likelihood that an effect of density existed but failed to be detected. The ranges of adult densities used in these experiments, 0–28, 0–56, 0–112, and 0–112·m^{-2} more than bracket the range of densities found in the sampling at any site. Consequently, no correc-

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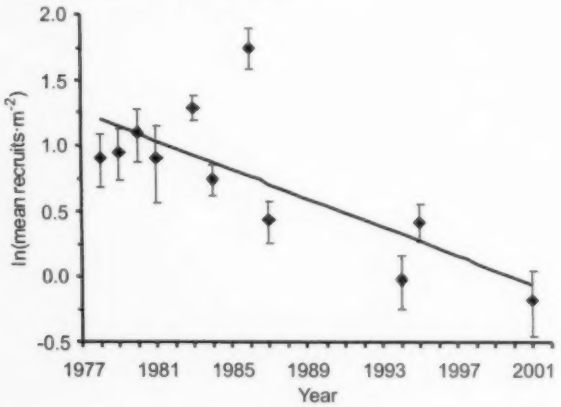
Table 2. Results of ANOVA tests of whether adult hard clam density inside 1-m^2 plots affects hard clam recruitment inside those plots.

		Recruits ≤15 mm*			Recruits ≤26 mm		
Source of variation	df	MSE	*F*	*p* value	MSE	*F*	*p* value
1978 Density	3	0.04	0.12	0.95	1.00	0.26	0.85
Error	15	0.37			3.80		
Total	18						
1979 Density	4	0.93	1.55	0.22	10.3	1.52	0.23
Error	24	0.60			6.82		
Total	28						
1980 Density	2	0.03	0.07	0.93	0.42	0.09	0.92
Error	8	0.49			4.90		
Total	10						
1981 Density	3	0.36	1.25	0.34	13.7	2.40	0.12
Error	11	0.28			5.7		
Total	14						

Note: ANOVA notation: df, degrees of freedom; MSE, mean square error; *F*, *F* ratio; *p* valve, probability.
*Recruits ≤15 mm were transformed by $\ln(x + 1)$ before analysis to meet homogeneity of variance assumption by Cochran's test at $\alpha = 0.05$ (transformation was unnecessary for recruits ≤26 mm).

Fig. 2. Temporal trend in annual mean recruitment in hard clams (*Mercenaria mercenaria*) within the Rachel Carson NOAA National Estuarine Research Reserve Sanctuary in Middle Marsh, Back Sound, N.C. Each plotted point represents ln(mean number of recruits <26 mm in length) present in October per replicate 1-m^2 plot. Error bars represent ln(mean ± SE, standard error). Recruits can be defined by clams <15 mm or <26 mm in length (based on data in Peterson et al. 1983). Using the more restrictive definition (<15 mm) yields the qualitatively identical regression outcome. Regression equations for full data sets using all samples are as follows. 15-mm recruits: $\ln(Y) = 0.920 - 0.0214X$, $R^2 = 0.050$, $n = 300$, $p < 0.0001$; 26-mm recruits: $\ln(Y) = 1.251 - 0.0316X$, $R^2 = 0.100$, $n = 300$, $p < 0.0001$, where Y is number of recruits·m^{-2} + 1 and X is year – 1978. The actual data illustration plots annual mean recruit density because the large number of coincident sample results makes a plot of all points unreadable. The regression equation for the annual means (<26 mm) is $\ln(Y) = 1.20 - 0.0547X$, $R^2 = 0.525$, $n = 11$, $p = 0.011$, where Y is mean density of recruits·m^{-2} and X is year – 1978.



tion of recruitment counts for local density of adult clams inside enclosures is justified.

Linear regression analyses on $\ln(x + 1)$-transformed recruitment counts from each sample plot, done to test whether recruitment of hard clams varied directionally over time, revealed highly significant ($p < 0.0001$) declines over the 24-year period (Fig. 2). The significantly declining temporal trend in recruitment was consistent independent of choice of size limit to define recruits, 15 mm or 26 mm. Using the regression equations to provide estimates of the magnitude of reduction in recruitment reveals declines from 1978 to 2001 of 65% in average numbers of recruits defined by a 15-mm length limit and 72% for recruits defined by a 26-mm limit. Year explained only a small fraction (5–10%) of total variance in hard clam recruitment among 1-m^2 samples because of spatial variability, so the long-term data record was critical to the ability to establish significance in the trend. Figure 2 plots the analogous regression through the annual mean recruit densities as illustration of the trend because coincidence of points in the full 300-sample data set prevents visual depiction. Even the regression across the annual means is significant at the 0.05 level (Fig. 2; $p = 0.011$) with sample size of 11. About 53% of the variance in this regression over annual means is explained by year, demonstrating by contrast to the full regression model how much spatial variability contributes to variation among 1-m^2 samples.

Temporal changes in hard clam density and spawning stock biomass over the 18+-year period from winter 1980 until autumn 1997 are evident from our fishery-independent sampling of three different habitats (Table 3). In the muddy sand tidal tributary at Johnson Creek in Core Sound, the average density of hard clams declined by 95% from winter 1980, before the area was subjected to intense harvest (Peterson et al. 1983), to autumn 1997 (Table 3). Over this same time period, the sand flat at Baldhill Bay in Back Sound exhibited an estimated decline of 17% in hard clam density, whereas the nearby seagrass bed at Twelve O'Clock Shoal experienced an estimated decline in hard clam abundance of 79%. The age-frequency distribution at Johnson Creek had differed historically from those of the sand flats and seagrass beds of Back Sound by possessing more older clams: the new age data (Table 3) are based on far fewer clams but appear to reflect a continuing contrast. Intense exploitation of

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Table 3. Changes in hard clam density, age distribution, and springtime gonadal mass production per 0.25 m^2 over an 18-year period at three sites in Back and Core Sounds, N.C.

	Percentage of the sample	
Year class (year)	Winter 1980*	Autumn 1997
Muddy sand creek: Johnson Creek in Core Sound		
<1	6	0
1	4	0
2	6	0
3	8	0
4–10	47	67
>10	29	33
Mean density per 0.25 m^2 ± SE	1.59 ± 1.28 (n = 34)	0.08 ± 0.05 (n = 36)
Mean gonadal production (g) per 0.25 m^2	1.15	0.19
Sand flat: Baldhill Bay in Back Sound		
<1	40	25
1	14	17
2	15	25
3	4	25
4–10	15	8
>10	12	0
Mean density per 0.25 m^2 ± SE	0.40 ± 0.05 (n = 216)	0.33 ± 0.10 (n = 36)
Mean gonadal production (g) per 0.25 m^2	0.100	0.075
Seagrass bed: Twelve O'Clock Shoal in Back Sound		
<1	23	0
1	18	30
2	26	20
3	13	0
4–10	14	40
>10	6	10
Mean density per 0.25 m^2 ± SE	2.26 ± 0.24 (n = 216)	0.48 ± 0.15 (n = 21)
Mean gonadal production (g) per 0.25 m^2	0.814	0.377

Note: SE, standard error.
*1980 Data for Johnson Creek from Peterson et al. (1983) and for Baldhill Bay and Twelve O'Clock Shoal from Peterson et al. (1984, 1987).

the very old hard clams at Johnson Creek did not detectably enhance recruitment there or natural restoration of the population (Table 3). Estimates of temporal change in spawning stock biomass of hard clams largely reflect the patterns exhibited by clam density because the size distributions that also contribute to gonadal production (Peterson 1983) did not change dramatically over the 18 years (Table 3). Consequently, spring spawning stock biomass declined by an estimated 24–46–83% (depending on site) from the years of 1980 to 1997 (Table 3).

Discussion

Given the typically high spatial variance in recruitment of infaunal bivalves and other soft-sediment benthic invertebrates (e.g., Olafsson et al. 1994), a long-term time series is critical to allow detection of trends over time. In addition, holding methodology and habitat stratification constant over time is important to allow rigorous testing of temporal trends. Because of our long-term research on hard clams in North Carolina, I was fortunate to possess historical data on recruitment using identical methods that allow me to evaluate the hypothesis that hard clam recruitment has declined over a 24-year period that coincides with increased demand and harvesting effort. Results of this analysis of temporal change in hard clam recruitment reveal a statistically significant and detectable decline of 65–72% from 1978 to 2001.

Fisheries statistics collected by the North Carolina Division of Marine Fisheries on hard clams (Fig. 3) illustrate the 5-fold increase in landings from 1976 to 1980, which has been associated with enhanced demand and increased harvest effort (Peterson et al. 1983). Much of this increase in effort in the central clamming district was achieved by fishermen rigging their boats for clam kicking, a mechanical harvesting technique that is far more efficient than hand harvest techniques (Guthrie and Lewis 1982). Unfortunately, no reliable and extensive systematic data were kept on fishing effort in the North Carolina hard clam fishery, so the landings data in Fig. 3 cannot confidently be corrected for effort to provide stock assessments. Nevertheless, a decline in landings by over 50% from the mid-1980s to present (2000) is evident and strongly suggests a stock declining from unsustainable levels of fishing mortality (Fig. 3). Moreover, these statistics on hard clam landings in North Carolina confound wild-caught clams with hatchery-raised aquaculture clams. Because the aquacultural production of clams in North Carolina has grown dramatically over the past 15–20 years, the decline in wild stock is even more dramatic than the decline in total landings plotted in Fig. 3. Although these landings reflect the entire state, not just the central region,

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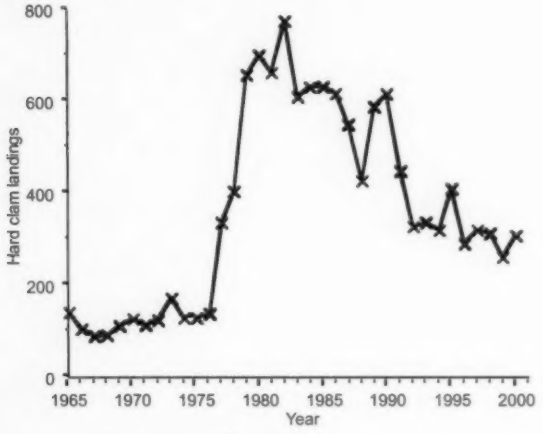
Fig. 3. Annual hard clam (*Mercenaria mercenaria*) landings (kg × 10^3) by commercial fishermen in North Carolina from 1965 to 2000 as recorded by the North Carolina Division of Marine Fisheries. These landings include both wild stock harvest and recently expanding aquacultural production from hatchery stock. Approximately 50% of the landings is derived from the region of Back Sound, Core Sound, North River, and Newport River within the hydrographic area that feeds larvae to Middle Marsh.



examination of the data for the Core Sound – Back Sound region alone reveals an analogous pattern over this time period. The Core Sound – Back Sound landings represent about half of the state's total and cannot be published separately because of a 20+-year-old confidentiality agreement still constraining use of the information.

Intense harvest of hard clams and other marine invertebrates is often advocated by the commercial industry by arguing that high adult abundance suppresses recruitment, such that compensatory settlement processes will follow intense harvest and rapidly replace the harvested animals. This expectation can be supported by hypothesizing a strong and negative effect of adults on larvae through filtration activities in the course of feeding. Our experimental manipulations of adult and juvenile hard clam density in 1-m^2 plots showed no evidence of such compensatory recruitment on a local scale. Furthermore, on a larger spatial scale, resampling of the Johnson Creek area about 15–20 years after severe depletion of a previously dense hard clam population revealed no strong recruitment in subsequent years and not even an initiation of recovery of historic abundance. Thus, our tests fail to support the operation of effective compensatory recruitment processes in the dynamics of hard clam populations on multiple scales of space and time.

To provide fishery-independent data on hard clam stock size and spawning stock biomass, I resampled three sites that represent different habitat types, a muddy sand creek, a shallow sand flat, and a seagrass bed, all of which I had sampled for hard clam abundance and size distributions years ago. Sampling was conducted in a fashion that held habitat type and condition identical between sampling years at each site. Because I had previously derived the prespawning relationships between clam shell length, age, and gonadal production (Peterson 1983), I used the density, size, and age data to also provide estimates of change in effective spawning stock biomass in this central region of North Carolina. The resulting density estimates exhibited declines of 17–95% from winter 1980 to autumn 1997, while spawning stock biomass (estimated as pre-spawning gonad mass per unit area) had declined by 24–83%. Consequently, these fishery-independent data confirm the large reduction in stock size since the mid-1980s that is implied by the landings trajectory. The combination of large declines in landings despite incentives of continued high demand, large reductions in stock size and spawning stock biomass in fishery-independent surveys, and large reductions in recruitment over this same time period strongly implies that recruitment overfishing is the cause of unsustainable yields of hard clams in the central district of North Carolina. Because both recruitment and spawning stock biomass decline with time, the implicit connection between them conceivably may be fortuitous rather than causal. Other factors may be changing with time and driving the observed reduction in hard clam recruitment. The most obvious change between the early 1970s and the next two decades is a warming of the climate. This could have direct or indirect impacts on hard clam recruitment, but hard clam populations extend much further south than North Carolina so impacts of environmental warming are not as compelling an explanation as recruitment overfishing.

While bivalve mollusc fisheries, and those for marine invertebrates more generally, are seldom managed to conserve spawning stock biomass, there is accumulating evidence to suggest that marine invertebrates can suffer recruitment limitation despite their high fecundity and complex life cycle. Gaines and Roughgarden (1985) have shown a recruitment shadow effect for a barnacle, in which increasing distance from the source of pelagically developing larvae results in progressively declining numbers of recruits. Limpets with planktonic larvae exhibit a pattern of enhanced abundance outside the boundaries of a marine reserve in the Canary Islands, indicative of population control by recruitment limitation (Hockey and Branch 1994). Florida spiny lobsters (Lipcius et al. 1997) and queen conchs (Stoner and Ray-Culp 2000) exhibit patterns of recruitment that strongly suggest the importance of spawning stock in driving population abundance of adults. In North Carolina, transplantation of adult spawners was apparently instrumental in restoring the bay scallop population and fishery in a water basin that had been depleted of scallops during a red tide outbreak (Peterson and Summerson 1992; Peterson et al. 1996). Brood stock transplants in California (Tegner 1993) and correlative patterns in Australia (Prince et al. 1988) provide analogous evidence that various abalones exhibit reduced recruitment when spawning stock biomass has been depressed. Sea urchin populations are commonly modeled using assumptions of Allee effects, where increased adult density enhances fertilization success and juvenile survival among protective spines (Quinn et al. 1993; Pfister and Bradbury 1996). Consequently, concern over possible impacts of adult spawning stock on subsequent recruitment of exploited marine invertebrates is justifiably growing.

Consideration of novel management actions to restore and sustain high harvest levels in the hard clam fishery has broad

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implications for other marine invertebrate fisheries. Establishment of spawner sanctuaries in each hydrographically defined water basin may represent an effective precautionary policy against overexploitation of spawning stock biomass (e.g., Tegner 1993). This represents a type of marine reserve (Quinn et al. 1993; Roberts 1997), but one that could differ substantially from what is typically contemplated. For example, polluted areas closed to bivalve harvest because of risk of transmission of human viral and bacterial diseases have inadvertently played this role as unplanned spawner sanctuaries. Stocking a planned network of spawner sanctuaries is also feasible for hard clams because of their longevity and lack of reproductive senility (Peterson 1983). Alternatively, establishment of fully protected estuarine reserves to include the function of providing a sanctuary network for hard clam spawning stocks could benefit multiple fisheries simultaneously as well as protect both habitat and the broad ecosystem basis for many fish and wildlife species (Roberts 1997; Allison et al. 1998).

An ideal opportunity for establishing a set of marine reserves is provided by restoring oyster reefs as part of shellfish enhancement programs. Because shell substratum provides protection from crab predation on recruiting hard clams (Peterson et al. 1995), hard clam abundance in restored oyster reefs in high-salinity estuaries is typically greatly enhanced. The importance of oyster reefs, now grossly depleted and degraded in part through application of damaging oyster dredges (Rothschild et al. 1994; Lenihan and Peterson 1998), as habitat for a diverse and economically important suite of finfishes and crustaceans implies that establishment of a network of oyster reef sanctuaries may serve an important role in sustaining a broad suite of coastal fisheries (Lenihan et al. 2001). However, after oysters themselves have been depleted on restored oyster reefs and after the reef matrix has been mined by habitat-damaging gear, the typical management practice in North Carolina and other states is to allow clammers to remove the hard clams by further destruction of the reef (e.g., Lenihan and Michelli 2000), which could otherwise serve as a hard clam spawner sanctuary.

Traditional methods of fisheries management, regulating effort through gear restrictions, harvest amount, size restrictions, and seasonal openings so as to sustain spawning stock biomass of wild stocks, have proven difficult to enforce and ineffective in most fisheries (Botsford et al. 1997). Rotating of open and closed areas, all within the same hydrologic water basin, may represent a more readily enforceable method of preserving spawning stock biomass in hard clams and other exploited sessile invertebrates (Fogarty and Murawski 1998) and is used to a limited degree for effort management in the hard clam fishery. Nevertheless, such a management scheme requires knowledge and care to preserve adequate spawning stock: rotation alone does not guarantee prevention of recruitment overfishing (Pfister and Bradbury 1996). Because hard clams reach reproductive maturity before the size of harvest for market, promotion of hard clam aquaculture could provide the benefit of enhancing recruitment to broad areas of the water basin and thereby sustaining otherwise unsustainable high levels of harvest of wild stocks. Furthermore, hard clam stocks have been shown capable of economically viable reseeding through introduction of hatchery-raised seed clams when introduced at sufficiently large size and in structured habitat like shell bottom to reduce risk of blue crab predation (Peterson et al. 1995). In some fashion, managers of exploited bivalves and other marine invertebrates must reflect with their management actions the real potential for recruitment overfishing. Consequences of insufficient attention to sustaining spawning stock of hard clams in North Carolina provide a warning of the general threats to the sustainability of invertebrate fisheries.

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Estimating the relative roles of dispersion and predation in generating regional differences in mortality rates of larval radiated shanny (*Ulvaria subbifurcata*)

P. Pepin, J.F. Dower, J.A. Helbig, and W.C. Leggett

Abstract: We followed three patches of larval radiated shanny (*Ulvaria subbifurcata*) using drogued drifters to assess regional differences in growth and mortality rates. We estimate dispersive losses directly using local surveys of surface currents corrected for the tidally induced divergence caused by the lack of synopticity of our surveys. We also monitored the abundance of juvenile capelin (*Mallotus villosus*), the major planktivore in the region, using hydroacoustic integration. The pattern of divergence–convergence showed considerable temporal and spatial variability, which resulted in substantial changes in the inferred biological mortality rates. The net mortality rates appeared to be directly proportional to the local abundance of pelagic fish. As with other patch studies, we are limited in the number of observations we obtained, but the results of this multidisciplinary study do suggest a strong potential for prey–predator interactions between larvae and adult planktivorous fish.

Résumé : L'utilisation de dériveurs à ancre flottante nous a permis de suivre trois « taches » de larves d'Ulvaires deux-lignes (*Ulvaria subbifurcata*) pour déterminer les différences régionales de leurs taux de croissance et de mortalité. Les pertes dues à la dispersion sont estimées directement à partir de l'inventaire local des courants de surface, corrigés pour éliminer la divergence induite par la marée due au manque de synopticité de nos inventaires. L'intégration hydroacoustique nous a de plus permis de suivre la densité des jeunes Capelans (*Mallotus villosus*), les planctonophages principaux de la région. Le pattern de convergence–divergence accuse une importante variabilité temporelle et spatiale, ce qui entraîne des changements considérables dans les taux estimés de mortalité biologique. Les taux nets de mortalité semblent être directement proportionnels à l'abondance locale de poissons pélagiques. Comme c'est le cas dans les autres études sur les « taches », le nombre d'observations que l'on peut obtenir est limité, cependant notre étude multidisciplinaire laisse croire qu'il y a une forte probabilité de relations prédateurs–proies entre les larves et les poissons planctonophages adultes.

[Traduit par la Rédaction]

Introduction

Despite nearly a century of extensive field and laboratory studies, our understanding of the factors regulating the high mortality rates of fish eggs and larvae remains far from complete (Leggett and Deblois 1994). The effects of food deprivation, predation, and advective losses have all been identified as potentially important factors. However, relatively few studies have applied a truly multidisciplinary approach to investigate the role of these interacting factors on population dynamics. Often, only a single factor is considered or there is insufficient contrast among cohorts to permit partitioning of the larval loss terms. Alternatively, variations in environmental conditions are often overlooked because field studies are performed at the population level (or over protracted time periods), thereby averaging conditions over scales larger than those that are relevant to larval prey–predator dynamics.

Patch studies of larval fish are designed to study variations in either growth or mortality rates that occur at small spatial and short temporal scales. By dealing with small elements of a larger population, the intent is to focus on the response of larvae on time scales that are more relevant to the dynamics of organisms with high turnover rates. The

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P. Pepin[1] **and J.A. Helbig.** Department of Fisheries and Oceans, P.O. Box 5667, St. John's, NF A1C 5X1, Canada.
J.F. Dower.[2] Department of Earth and Oceans Sciences, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
W.C. Leggett. Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada.

[1]Corresponding author (e-mail: pepinp@dfo-mpo.gc.ca).
[2]Present address: Department of Earth and Ocean Sciences, P.O. Box 3055, Cunningham Building, University of Victoria, Victoria, BC V8W 3P6, Canada.

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small spatial scale of the study site allows for more accurate and frequent measurements of the environmental conditions for the subpopulation of interest. Similar arguments apply to regional studies of small bays (Taggart and Frank 1990; Pepin et al. 1995).

Protocols used in patch studies generally consist of identifying an aggregation of larvae, delineating the extent of the local distribution as well as the centre of mass, which is then marked with a single drogue to follow drift. There are two fundamental elements needed when dealing with field estimates of mortality: monitoring population abundance over time to measure total losses and estimating the contribution owing to dispersal (advection is often, but not always, considered to be well measured using the drifter). Removing dispersive losses from the total change in population numbers leaves a residual mortality that is considered to reflect biological processes that can subsequently be related to prevailing environmental conditions.

With few exceptions (e.g., Fortier and Leggett 1985), dispersion is estimated using changes in the spatial distribution of the larvae while assuming a simple advection–diffusion–mortality model (e.g., Heath and MacLachlan 1987; Kim and Bang 1990). The underlying assumptions are that changes in the distribution of larvae provide an unbiased measure of dispersal and that biological mortality (e.g., predation) is spatially uniform. Consequently, estimates of dispersion and biological losses are not independent. It is therefore difficult to accurately establish the relative importance of biological and physical processes on perceived changes in population abundance.

From a logistic perspective, patch studies require intensive and frequent sampling over relatively short time scales. The duration of the experiment is also constrained by the need to obtain measurable changes in abundance to estimate mortality rates. Moreover, the degree of cohesion of the subpopulation places further restrictions on the duration of the study because the dispersal of larvae may limit how readily changes in state (e.g., feeding, growth, condition) and abundance can be detected. This limitation is further exacerbated when changes in the abundance and distribution of animals are used to estimate dispersion, because this can only be done reliably when the subpopulation is relatively well isolated from other such units by areas with very few larvae (i.e., lest other larvae be carried into the patch from the surrounding waters). To perform another experiment therefore requires locating another isolated unit, which explains why most reported patch studies of larval fish effectively sample only one unit.

Herein, we present the results of a study in which we followed three patches of larvae using drogued drifters to assess regional differences in growth and mortality rates. Our approach is novel relative to previous studies in that we estimate dispersive losses directly using local surveys of surface currents measured with an acoustic Doppler current profiler (ADCP, RD Instruments, San Diego, Calif.) while also monitoring the larvae, their prey, and their potential predators. Previous studies have not provided direct measurements of dispersion (except Fortier and Leggett 1985) and none has reported on the relationship between biological losses and predator abundance.

Materials and methods

Our objective was not to track an isolated patch of larval fish per se but rather to monitor larvae in proximity of a drifter for periods of 3–5 days while surveying changes in circulation, food density, and predator abundance. The experiment was repeated in three bays over a two-week period. At each site, our goal was to measure changes in larval abundance, dispersion caused by currents, and the abundance of planktivorous fish in the area (as potential predators).

Field work was carried out in three deep embayments along the east coast of Newfoundland between 23 July and 05 August 1997 (Fig. 1). The three bays sampled, Bonavista Bay (48°45′N, 53°20′W), Trinity Bay (48°00′N, 53°30′W), and Conception Bay (47°45′N, 53°00′W), are all part of the larger Labrador Northeast-Newfoundland Shelf, a physically dynamic system that is strongly influenced by the inshore arm of the Labrador Current, and wind forcing with a characteristic time scale of 5–15 days during the summer (deYoung and Sanderson 1995; Pepin et al. 1995). The Labrador Northeast-Newfoundland Shelf ecosystem is characterized by a subarctic Atlantic plankton community (Davis 1982; Laprise and Pepin 1995).

Survey design

The location of each study site was based upon obtaining repeated good catches of larval fish determined from oblique tows (0–40 m) using a 4-m^2 Tucker trawl equipped with sections of 1000, 570, and 333 µm mesh nitex (Pepin and Shears 1997). Because of patterns in overall abundance, we focused our study on larvae of the radiated shanny (*Ulvaria subbifurcata*). Once selected, the water mass was marked using a buoy attached to a 10-m holey-sock drogue located at a depth of 10 m (range 5–15 m). The drifter's position was monitored frequently (1- to 4-h intervals) using the ship's radar linked to a global positioning satellite system (GPS) and by visual confirmation. Each experiment was started between 22:00 and 02:00 local time. By following the drifter during each experiment, we also obtained a measure of advection of the water mass.

Following drifter deployment, a series of plankton tows along a square grid of nine stations was performed to obtain an estimate of population abundance. Stations were located 6.5 km apart along both north–south and east–west axes with the drifter at the centre station. This grid of stations was repeated every night (after 22:00 local time) during each experiment with station locations adjusted for drifter movement. Each survey took 7–8 h to complete. Plankton sampling was conducted at night to reduce potential bias associated with net avoidance by the larvae. At each station, we lowered a Seabird-25 CTD (sampling rate of 8 Hz, Seabird Electronics Inc., Bellevue, Wash.) to within 5 m of the bottom at a rate of 1 $m \cdot s,^{-1}$ after which we performed an oblique tow (0–40 m) for approximately 15 min using a 4-m^2 Tucker trawl. On deck, the net was washed and the sample was preserved in 95% ethanol. Ichthyoplankton were subsequently sorted (post cruise) and identified to the lowest taxonomic level possible. The length–frequency distribution for each species and sample was estimated by measuring up to 200 larvae. Standard length was measured to the nearest

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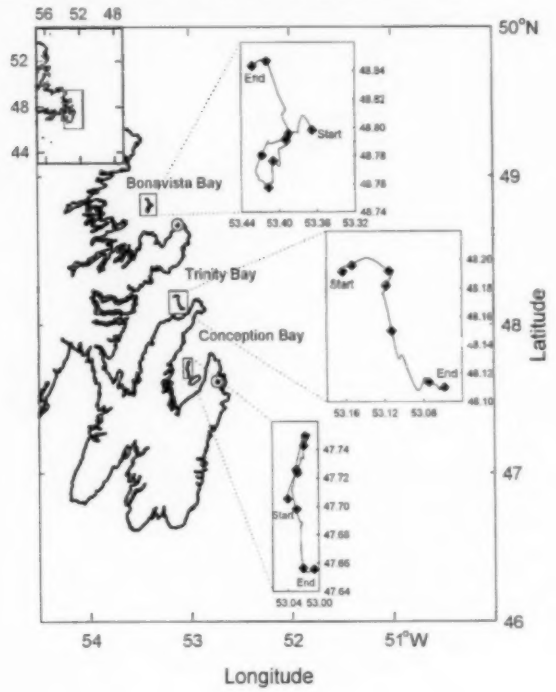
Fig. 1. Map of study region on the east coast of Newfoundland, Canada, showing the three study sites and the drifter tracks, with nodes at midnight and noon on each day following deployment of the drogue shown in the insets for each drifter track. The location of the St. John's Airport and Bonavista Lighthouse atmospheric stations are represented by shaded circles.



millimetre using a dissecting microscope and a gridded background.

On the first evening following drifter deployment (as well as each subsequent evening until the end of an experiment), we conducted a combined hydroacoustic survey of the region around the drifter using a hull-mounted ADCP (153 kHz) to measure currents and an EK500 echosounder (38 kHz, Simrad AS, Norway) to measure the abundance of pelagic fish. This survey was conducted from 18:00 to 21:00 local time along an octagonal track totalling 45 km in length (each side ~5.6 km) centred on the drifter's location. The survey path was designed to provide an estimate of current divergence for the study site and the duration was aimed at minimizing error caused by variations in tidal currents. By integrating flow normal to the survey path over the entire track, we obtained a measure of the divergence over the study site (Appendix A). Data from the ADCP, operated in bottom track mode, were recorded in 4 m depth bins (first bin at 7–11 m). A sampling interval of two minutes was used to give a nominal ensemble average based on at least 50 pings. The standard deviation of unsmoothed single bin currents was ~0.03–0.05 $m \cdot s^{-1}$. Data from the EK500 were recorded for depth bins of 10–25, 25–40, 40–55, 55–75, 75–100, and 100–200 m using 3-min integration intervals with a threshold of –58 dB. The composition of echoes was verified by performing an oblique tow (0–100 m) using an International Young Gadoids Pelagic Trawl (IYGPT) for 20 min at a speed of 2 $m \cdot s^{-1}$. All fish caught were counted and identified to species with up to 200 individuals from each species being measured for standard length to the nearest millimetre.

Otolith microstructure analysis

Prior to otolith extraction, each animal was assigned a unique identifier number and measured for standard and total length (to the nearest 0.1 mm). Body measurements of larval *U. subbifurcata* used for otolith microstructure were made with an Optimas® image analysis system (V.4.1, Media Cybernetics, Inc., Silver Spring, Md.) connected to a dissecting microscope. Lapillar and sagittal otoliths were extracted from 509 larvae (up to 10 larvae were selected at random from each station). Each otolith was mounted in a small drop of Crystal Bond® thermoplastic cement placed on a microscope slide. All subsequent analyses were performed using the sagitta, choosing the right or left otolith at random unless one proved impossible to read. Each otolith was ground to the mid-plane using 0.3-µm lapping film. An Olympus BH-2 compound microscope (500× magnification) and Optimas imaging system were used for all otolith measurements. Minimum and maximum radius, as well as the hatch check, were measured (in µm) relative to the nucleus of the otolith. Total area (μm^2) was estimated by identifying the periphery of the otolith. The operator then measured the width of each increment by marking the outer edge of that increment along the longest axis of each otolith, which occasionally required refocusing of the image. Analyses of patterns in increment widths were performed using data standardized to zero mean and unit standard deviation ($z_{i,j} = (x_{i,j} - \bar{x}_j)/s_j$, for specimen i in age interval j) because of the covariance between mean increment width and increasing age (Pepin et al. 2001). Throughout the analysis we consider that each increment represents one day (A. Bochdansky, Department of Biology, Queen's University, Kingston, Ont., personal communication).

Comparison of the age–length relationship among sites was performed using an analysis of covariance (ANCOVA). Comparisons of patterns of increment widths was accomplished using a repeated measures multivariate analysis of variance (MANOVA) using the information of the last three increments deposited before capture of a larva. Our choice to use the last three increments was based on Pepin et al.'s (2001) analysis, which showed strong serial correlation over a period of three days. Age-dependent patterns in the cumulative probability distribution of standardized increment widths were determined using nonparametric local density estimators (Pepin et al. 1999).

Results

Winds during the study period were generally from the south–southwest at speeds less than 10 $m \cdot s^{-1}$ (Fig. 2). Winds from both St. John's airport and the Bonavista lighthouse exhibited similar patterns of variation with wind speeds being somewhat smaller at the former location (Fig. 2). During the first drifter deployment, winds were relatively constant until the last 12 h, when they shifted to northeasterlies. Weak and

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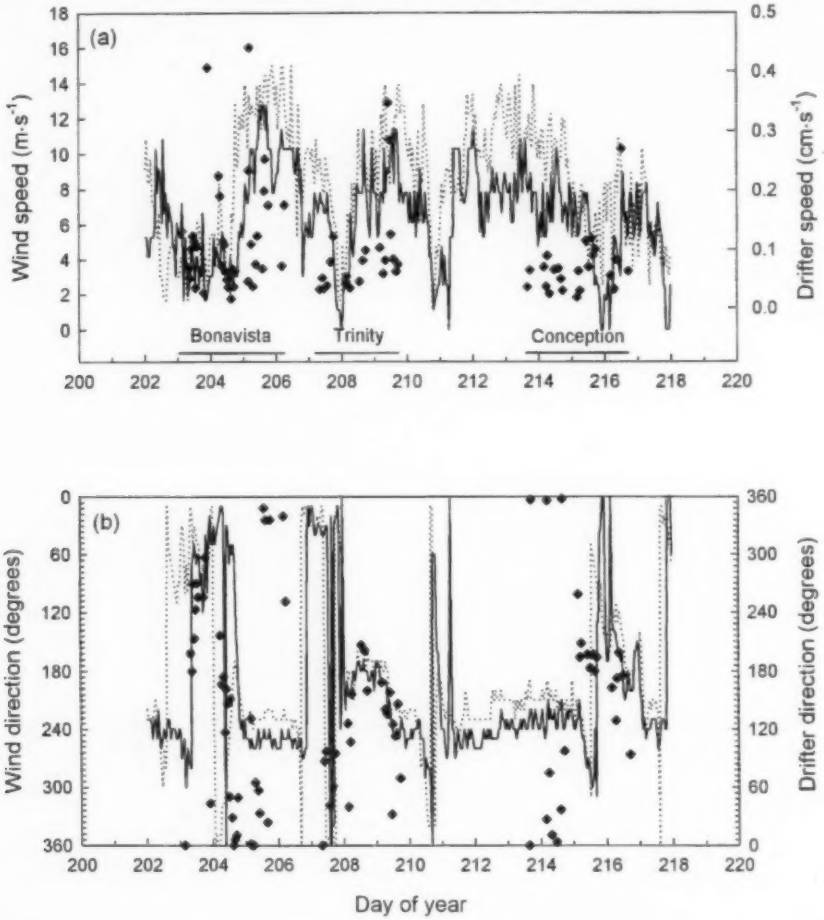
Fig. 2. (*a*) Wind and drifter speed and (*b*) direction during the study period. Dotted and solid lines represent the wind features measured at the Bonavista Lighthouse and St. John's Airport, respectively. Diamonds represent the drifter speed and direction measured during each observation period. Wind speed and direction are referenced on the left *y* axis, whereas those for drifters are on the right *y* axis. Note that the scale of the wind direction axis is opposite to that of drifters to reflect that meteorological services report winds from their source, whereas current direction refers to the direction of motion. The horizontal bars in (*a*) indicate each deployment period.



variable winds (~5 m·s^{-1}) dominated during the second and third deployments (Fig. 2).

Movement of the drifters appeared to be independent of wind direction or strength. Although the wind blew predominantly to the northeast, drifters tended to move in a southerly direction for most of the period of study at the Trinity and Conception Bay sites (Fig. 1). Drifter movement in Bonavista Bay was variable but largely independent of wind speed and direction (Fig. 2). The overall movement of the drifter at each of the study sites was much less than the total area of the initial survey grid. Average drift speed was 11.6 cm·s^{-1} (standard deviation (SD) = 9.7) in Bonavista Bay, 9.0 cm·s^{-1} (SD = 7.9) in Trinity Bay, and 7.2 cm·s^{-1} (SD = 5.1) in Conception Bay. The general behaviour of each drifter was consistent with the pattern of currents measured using the hull-mounted ADCP. Likewise, the average current speeds were similar.

Surface currents (7–31 m depth) measured along the daily circuit of each study area showed considerable daily variation in strength and direction. However, within any one evening, the circulation pattern showed a reasonably smooth progression around the periphery of the study zone. The observed divergence at all sites showed considerable temporal variability, as did the tidally induced divergence that is associated with the lack of synopticity in our survey of currents around the drifter (Fig. 3). In all instances, the tidally induced divergence was of the same order as, or slightly greater than, the observed values, but the direction was not necessarily the same. As a result, the net divergence showed substantial departures from the instantaneous observations. At all three sites, the current observations indicate that the initial conditions that allowed us to identify patches of larvae were the result of a net convergent flow, but that the subsequent transport resulted in substantially different patterns of plankton dispersal from the area around the drifter. In Bonavista Bay, the net divergence increased substantially during the course of the study, more than would have been inferred without the correction for the tidally induced diver-

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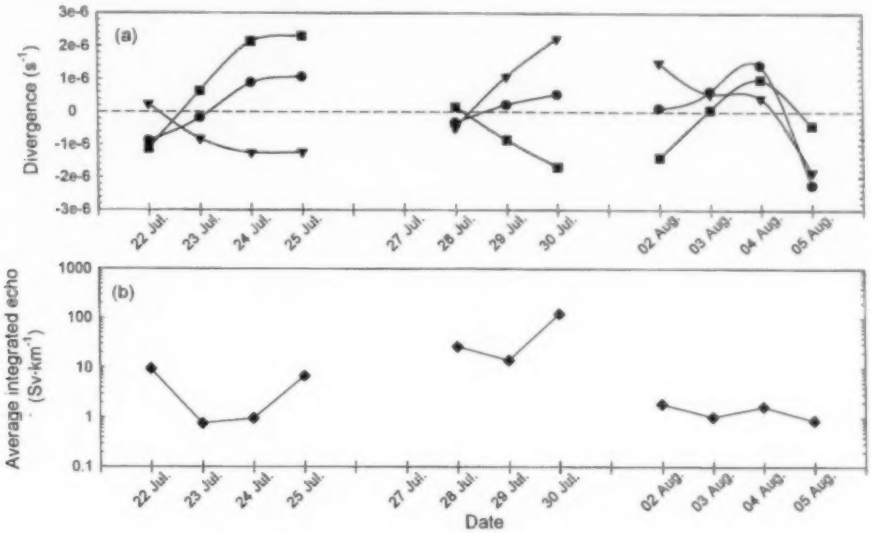
Fig. 3. Environmental time series of the observed (circles), tidally induced (triangles), and net divergence (squares), measured as the integrated flow normal to (*a*) the survey circuit and (*b*) the average integrated hydroacoustic backscatter, at the three study sites. The period of 22–25 July represents the study in Bonavista Bay, the period 27–30 July represents Trinity Bay, and the period 2–5 August represents Conception Bay.



gence. In Trinity Bay, the pattern of net divergence indicated an overall convergence of water and larvae into the study area. Conception Bay had the most variable pattern of flow, changing from one of convergence to one of divergence and back to convergence while we tracked the drifter.

Hydroacoustic data from the nightly circuits around the study area showed strong distinctions between the three sites. The majority (>97%) of targets were juvenile capelin (*Mallotus villosus*) between 8 and 18 cm in length. The average integrated echo density was highest in Trinity Bay, followed by Bonavista and Conception bays (Fig. 3), with approximately an order of magnitude difference between the first two sites and a half order of magnitude difference between the latter two.

Changes in population abundance in Bonvasita and Trinity bays were significantly different from 0 ($P < 0.05$) based on a paired *t*-test comparison of the length–frequency distributions. Changes in the abundance of larvae in Conception Bay were not significantly different from 0 ($0.2 > P > 0.1$). We nevertheless contrasted the estimated mortality rates among the three sites, since our objective was to assess the relative contributions of biological and physical losses on our perception of changes in population abundance. Average mortality rates, prior to correction for dispersive losses, were highest in Trinity Bay, lowest in Conception Bay, and uncorrelated with the average divergence at each site (Fig. 4). The overall variation in uncorrected mortality rates among sites was approximately fivefold. The overall effects of divergent currents on population estimates were almost negligible in Conception Bay, whereas they were approximately equal in magnitude in Bonavista and Trinity bays, although divergence caused a population loss in the former while convergence lead to a population increase in the latter. Population losses owing to dispersal will lower the net mortality rate from the observed, whereas a population increase will cause the opposite effect. Overall, there was a 11-fold difference in the net larval mortality rates between the three sites. The net mortality rates followed the same order as the hydroacoustic estimates of capelin abundance, with the lowest mortality rates observed in the area having the lowest integrated hydroacoustic returns and the highest mortality rates observed in the area of highest capelin abundance (Fig. 4). The pattern appears to follow a linear trend of mortality rates in relation to integrated echos but with only three observations; fitting a functional relationship is inappropriate. There is also evidence of size dependency in mortality rates. Estimates of mortality rates for each of the 1 mm length classes were derived for each site. Although the pattern at each site is relatively noisy because of the limited number of stations, the average size-dependent mortality estimates indicate that biological mortality rates increase with increasing length (Fig. 5). There is some indication of production (i.e., positive mortality values) in the smaller size categories (possibly owing to emergence). Loss of the larger categories (>15 mm) may have been due to settlement, since shanny larvae are known to settle at lengths of 16–18 mm (LeDrew and Green 1975). However, the increase in mortality with increasing length remains relatively steady.

The average daily growth rates of larvae, based on an age–length relationship, were highest in Conception Bay (0.42 mm·day^{-1}, standard error (SE) = 0.012) followed by Bonavista (0.37 mm·day^{-1}, SE = 0.0097), and Trinity (0.35 mm·day^{-1}, SE = 0.013) bays (Fig. 6). The latter two growth rates are not significantly different from one another but both are significantly different from that observed in Conception Bay (ANCOVA, $F_{2,328} = 8.48$, $P < 0.01$). The standardized increment widths for the three days prior to capture showed a pattern that was consistent with that of the age–length relationship (MANOVA, $\lambda = 0.47$, $F_{6,482} = 36.6$, $P < 0.001$) (Fig. 7). Although there was some indication that

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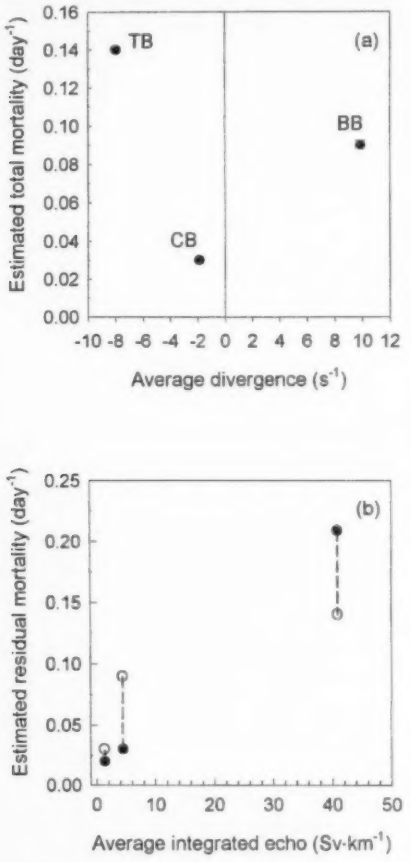
Fig. 4. (*a*) Total mortality rates of larval radiated shanny (*Ulvaria subbifurcata*) from each site (BB, Bonavista Bay; TB, Trinity Bay; CB, Conception Bay) prior to the correction for integrated dispersive losses attributable to transport, in relation to the average divergence measured during each study period. (*b*) The estimated net mortality (solid circles) is shown in relation to integrated hydroacoustic echo, a measure of the abundance of pelagic fish. The change from the observed mortality (open circles) attributable to divergence (or convergence) is shown by the broken lines.



the differences among bays were more pronounced in older age categories, the difference was not significant ($\lambda = 0.78$, $F_{69,720} = 0.89$, $P > 0.5$).

The pattern of current increment widths in each of the bays differs from what we observe when we contrast the growth at age between individuals from one site. Older individuals in Bonavista and Trinity bays have wider growth increments at age 5–7 than younger larvae (Fig. 8). In contrast, we find the opposite pattern in Conception Bay (Fig. 8), where younger larvae have higher growth rates at ages 5 to 7 than older individuals captured in that area.

Discussion

It has been difficult to reconcile the results from previous patch studies because each represents an independent biological (species, environment) and logistic (sampling scheme, frequency and collection) entity: a single patch is tracked over periods of a few days to a few weeks (Davis et al. 1991; Murdoch and Quigley 1994). In this study, we identified and followed three aggregations of radiated shanny larvae for periods of 3–5 days. We were able to observe the dynamic changes in the physical environment that allowed the formation and subsequent dispersal of the patches as the currents carried them, as well as to contrast the predator (this study) and prey (J.F. Dower and P. Pepin, unpublished data) environments of the different sites.

The circulation pattern at each of the study sites was initially characterized either by convergence (Bonavista and Trinity bays) or weak divergence (Conception Bay) that likely led to the formation of the aggregations that we originally identified with our sampling. Subsequent changes in the pattern of currents in the region resulted in different patterns of dispersal at each site. Changes in population abundance attributable to divergence or convergence were more substantive in Bonavista and Trinity bays than in Conception Bay. Total mortality rates were already lower in Conception and Bonavista bays relative to Trinity Bay, but variations in the contribution of dispersal by divergent currents to perceived losses resulted in greater differences in the rates of "biological" losses among the sites. However, instantaneous or average measures of divergence at each of the study sites could not explain the differences in mortality rates between sites that appear to be dominated primarily by other biological effects.

Estimates of dispersive losses from previous studies have ranged from ~0.016 to 0.3 day^{-1} and are highly scale dependent (summarized by Helbig and Pepin 1998). For an area of ~100 km^2, the estimates from this study (absolute values range from 0.02 to 0.13 day^{-1}) are somewhat lower than the value of 0.20 day^{-1} predicted from the functional relationship reported by Helbig and Pepin (1998), but similar in magnitude to that reported by Fortier and Leggett (1985). However, in contrast to previous observations, we found evidence of convergence within the study area. This difference may be due in part to the nature of the systems under study. Most previous work concentrated on egg and larval populations that occur in naturally advective systems with strong mean currents, where spawning and nursery grounds are separated. Circulation patterns in coastal Newfoundland bays are characterized by high variability in current speed and direction and a weak mean circulation (deYoung and Sanderson 1995; Davidson 1999). However, the difference may also be due in part to the approach taken, whereby we had direct observations of changes in the small-scale circulation features in our study area, and whereas most other studies rely on fitting a model that does not always provide details of the small-scale variations in the currents. Predicting the occurrence and intensity of areas of convergence or divergence remains one of the most difficult tasks of circulation models. Our results suggest that the ability to characterize variations in the current field is important in understanding the net effect of dispersal and transport on changes in the abundance of planktonic organisms.

The contrasting net mortality rates among the three sites exhibit a pattern of variation that follows that of the average hydroacoustic signal, an estimate of the abundance of pelagic fish. With only three observations, it is premature to

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Fig. 5. Average size-dependent mortality rate of larval radiated shanny (*Ulvaria subbifurcata*). The values shown represent the average calculated based on estimates from all three sites. There is some evidence of production in the smaller length categories.

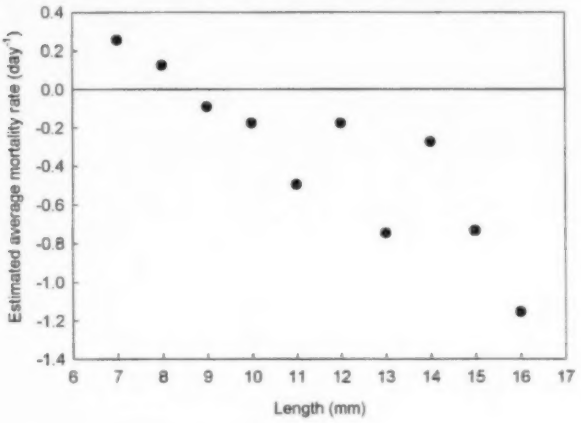
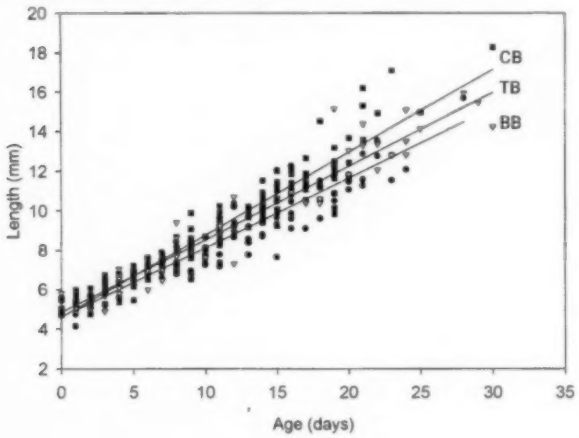
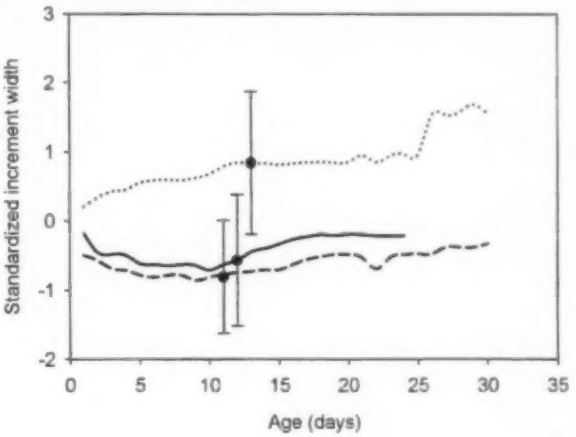


Fig. 6. Age–length relationships for radiated shanny (*Ulvaria subbifurcata*) larvae from the three study sites. The acronyms next to each regression lines identify the study site (BB, Bonavista Bay (solid circles); TB, Trinity Bay (open triangles); CB, Conception Bay (solid squares)).



identify a functional relationship between the rate of loss of the abundance of pelagic fish. Nonetheless, the signal we observed does suggest a linear relationship, consistent with observations from Smith et al. (1989), who found a linear relationship between the abundance of pelagic fish and the mortality rates of pelagic fish eggs estimated from population surveys. There is also further information that suggests that pelagic fish were likely to be important predators of larval fish during our study. In addition to the pattern of increasing average mortality with increasing pelagic fish abundance, we found that size-dependent mortality increased with increasing larval length. A recent summary of laboratory studies (Paradis et al. 1996) indicates that vulnerability of larvae to predators increases until the prey–predator length ratio reaches 0.1 (10%). At our study sites, juvenile

Fig. 7. Median standardized increment widths among the three sites (Bonavista Bay, solid line; Trinity Bay, dashed line; Conception Bay, dotted line) in relation to total age at increment deposition. The medians were estimated using nonparametric local density estimators using the last three increments deposited in each individual larva. The error bars are shown to provide a perspective of the general level of variation in increment widths among individual larvae. Error bars represent the average 10th and 90th percentiles of the distributions of standardized increment widths for each site.



capelin ranged in length from 8 to 18 cm, which would suggest that vulnerability of the larvae sampled during this study (4–14 mm) would have been increasing toward the peak for all length categories. This would have resulted in increasing mortality with increasing size of the *U. subbifurcata* larvae, in a manner consistent with our observations, if pelagic fish were the dominant predators. This is also consistent with modelling results of Paradis and Pepin (2001), who found that for several species of larval fish, pelagic fish had the greatest potential impact. The best approach would have been to obtain information on stomach contents of the predators to corroborate our inference about the role of juvenile and adult capelin in determining biological losses. However, we have been unable to obtain adequate samples to provide this information, partly because larval fish are rapidly digested in contrast to eggs (Hunter and Kimbrell 1980).

The patterns of growth rates and growth histories show contrasting patterns among sites. Larvae in both the Bonavista and Trinity Bay sites had low growth rates and daily increment widths when contrasted with those in Conception Bay. However, when we contrast the history of older individuals with their younger counterparts within a site, we find that individuals that grew more rapidly during their early stages of development were more likely to survive in the former two bays relative to the latter, where older individuals appeared to have lower growth rates relative to younger larvae. Comparing the three bays we see that differences in the median growth increment widths at ages 5 to 7 are most pronounced in younger larvae and least notable in older ones. The patterns of (growth) selective loss are not consistent with expectations based on vulnerability to predation by pe-

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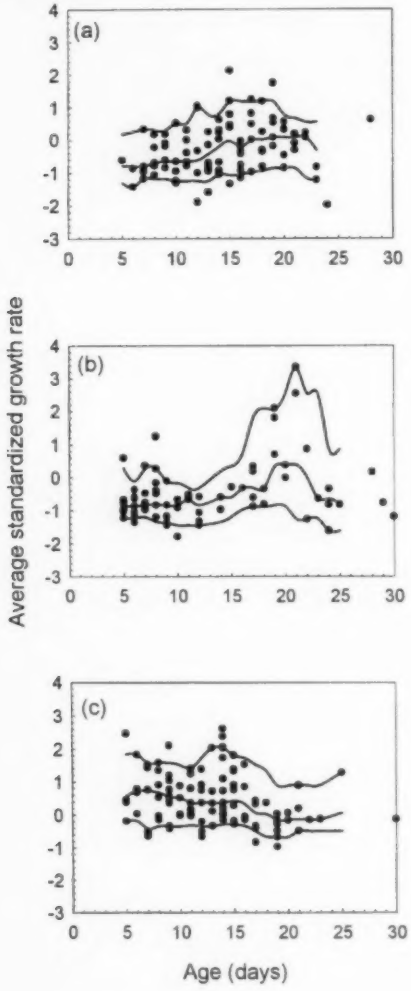
Fig. 8. Individual standardized growth increments at ages 5 to 7 in relation to the age of the larva at capture, for (*a*) Bonavista, (*b*) Trinity, and (*c*) Conception bays. The lines represent the 10th, 50th, and 90th percentiles of the distribution estimated using nonparametric local density estimators.



lagic fish. Although one would expect to have higher overall survival rates of faster growing individuals to settlement or recruitment (a transition or shift in behaviour or niche), patterns of loss within an environment or stage will depend on the cumulated impact of selective pressures. In the case of predation by pelagic fishes, vulnerability of radiated shanny larvae would likely have increased with increasing length (because of the size relative to that of the predator), thus making faster-growing individuals more likely to be eaten. However, the pattern we observe is opposite to what we would expect based on our short-term measures of mortality rates and environmental conditions. This is likely due to the pattern of variation in prey–predator interactions between larvae and active migratory fishes. In coastal Newfoundland, capelin migrate from south to north (Templeman 1948) and their appearance at one site and subsequent movement either within a bay or to other areas occurs on the time scale of a few days and is highly variable from year to year. Short-term observations at a single site (~100 km^2) are therefore unlikely to capture changes in predation pressure that have developed over periods of several days to weeks. Such mesoscale effects can only be described by following a population of larvae over an extended period of time (weeks), which is not possible for patch studies. Consequently, it is not entirely unexpected that recent growth histories and contemporary estimates of mortality rates are in contrast to one another. In fact, this further illustrates the need for caution in the interpretation of patterns in growth histories. Instantaneous observations of the correlation between larval fish and environmental conditions are unlikely to provide an accurate measure of the dynamic changes in either physical or biological conditions that have taken place at a given study site. Differences in growth rates among sites increased with both temperature and prey availability (J.F. Dower and P. Pepin, unpublished data), but the relationship was generally weak and measurements of environmental conditions may not accurately reflect individual growth histories (Pepin et al. 2000).

Short-term variations in larval transport and dispersal are known to influence the perceived rates of change in population abundance, particularly at small spatial scales (Taggart and Leggett 1987; Pepin et al. 1995; this study). However, by providing concurrent measurements of the potential advection or dispersal, we can directly estimate the contribution of currents to changes in population abundance with reasonable accuracy (Helbig and Pepin 1998). When it comes to understanding prey–predator dynamics, the spatial and temporal scales of interaction are more difficult to investigate.

We know from laboratory studies that the vulnerability of fish larvae to invertebrate carnivores tends to decrease as the larvae grow because of their relative sizes (see Paradis et al. 1996) and their spatial distributions tend to minimize overlap (Frank and Leggett 1982). In instances where there is a potentially significant overlap, we know that tracking changes in the distribution of a patch of both groups will likely provide some understanding of their interactions, since both larvae and invertebrate carnivores are planktonic and will thus be subject to similar physical forces. To date, invertebrates appear to have their greatest impact on larval fish in areas where the former are highly concentrated over large areas (Taggart and Leggett 1987; Cowan and Houde 1993; Purcell et al. 1994). In contrast, the potential influence of juvenile and adult planktivorous fishes as predators of larval fish is not as well understood. They are highly mobile, patchily distributed, and their changes in abundance at a given site are generally difficult to track over time. The dominance of high frequency variations in spatiotemporal abundance of such mobile predators places limitations on our ability to observe such interactions and to project their implications at the population level (Pepin et al. 2000).

The short-term multidisciplinary observations in this study have provided an indication of the potential significance of interactions between larval fish and pelagic fish under field conditions. As with all other patch studies, we are limited in the number of observations we obtained, but in contrast to previous work, we were at least able to obtain observations from several patches in similar ecosystems. Repeated short-

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term field studies of prey–predator interactions are needed to support the inferences derived herein. We must keep in mind that the highly dynamic nature of those interactions must be characterized if we are to make progress toward understanding the role of predation on the dynamics of the early life stages of fish.

Acknowledgements

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Appendix A.

We decompose the horizontal current $u(x,t)$ into its tidal and residual components

$$u(x,t) = u_t(t) + u_D(x)$$

and assume that the tide, $u_t(t)$, is space independent over the study area and the residual, $u_D(x)$, is time independent over the duration of the experiment and has divergence D. The divergence averaged over the study area is

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$$d = \frac{1}{A}\int_A (\nabla \cdot u)\mathrm{d}x = \frac{1}{A}\int_S u_n \mathrm{d}s + D$$

where S is the path enclosing A and u_n is the outward component of u_t normal to the path. The tidal component is divergence free because it is space independent, but it induces an apparent divergence because the survey is not instantaneous. Defining the survey path parametrically by the time, $\mathrm{d}s = c\mathrm{d}t$, where c is the ship's speed, we have

$$d = \frac{c}{A}\int_S u_n(t)\mathrm{d}t + D$$

The tide is given as the sum over its harmonic constituents as

$$u_n = \sum_{k=0}^{k=N} a_k \cos(\omega_k t + \phi_k)$$

where a_k and ϕ_k are the amplitude and phase of the kth constituent and ω_k is its frequency. For a single constituent, straightforward computation shows that the induced divergence is

$$d_k = \frac{a_k c}{w_k A}\left\{ s_0 - \left(1 - \frac{\sqrt{2}}{2}\right)(s_1 + s_4 - s_5) - \frac{\sqrt{2}}{2}(s_2 + s_3 - s_6 - s_7 + s_8)\right\}$$

where

$$s_k = \sin(\omega_k \Delta t + \phi_k)$$

and Δt is the time required to complete one leg of the survey octagon. The apparent divergence thus depends on the duration of the survey as well as the phase of the tide.

In the study area, the tide is dominated by the M2 component. For $a_k = 0.02$ m·s^{-1}, $c = 2.5$ m·s^{-1}, $\omega = 2\pi/12.5$ hours, and $A = 150$ km^2, the error varies over the range of $\pm 0.6 \times 10^{-6}$ s^{-1} depending on the tidal phase. This is of similar magnitude to the measured divergence. However, the variation in tidally induced divergence is nearly constant over each experiment's duration, so the overall effect is to shift the estimated divergence up or down uniformly.

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Using bioenergetics models to predict stable isotope ratios in fishes

Chris J. Harvey, Paul C. Hanson, Timothy E. Essington, Paul B. Brown, and James F. Kitchell

Abstract: To describe temporal dynamics of stable isotope ratios in fishes, we developed a bioenergetics-based model that links isotope ratios to growth, as influenced by fish size, temperature, diet, and prey quality. The model includes error terms for isotope ratios, diet proportions, and fractionation. The model accurately predicted temporal $\delta^{15}N$ dynamics of lake trout (*Salvelinus namaycush*) in a diet-switch experiment but was less successful for $\delta^{13}C$, possibly because of variable fractionation. The model was then used in three heuristic applications. In a diet-validation scenario, a model derived from limited knowledge of rainbow smelt (*Osmerus mordax*) diet reasonably estimated $\delta^{13}C$ and $\delta^{15}N$ compared with a null model but inaccurately estimated prey consumption. In a scenario where adult lake trout briefly cannibalized stocked lake trout fingerlings, the detectability of a cannibalism-induced $\delta^{15}N$ increase depended on predator size, duration of cannibalism, and sample size. In a scenario where seasonal isotopic variability occurred at the base of a food web, variation propagated to higher trophic levels depended on consumer size and diet. Our approach is most valuable when used to examine multiple diet combinations that produce observed stable isotope ratios; one can then identify the most reasonable diets through field tests or other observations.

Résumé : Dans le but de décrire la dynamique temporelle des rapports d'isotopes stables chez les poissons, nous avons mis au point un modèle basé sur la bioénergétique qui relie les rapports isotopiques à la croissance, elle-même influencée par la taille du poisson, la température, le régime alimentaire et la qualité des proies. Le modèle comprend des termes d'erreur pour les rapports isotopiques, les proportions du régime alimentaire et le fractionnement. Le modèle a permis de prédire avec précision la dynamique temporelle du $\delta^{15}N$ chez le Touladi (*Salvelinus namaycush*) dans une expérience de changement de régime; il a été moins efficace dans la prédiction de $\delta^{13}C$, peut-être à cause du fractionnement variable. Le modèle a aussi servi dans trois applications heuristiques. Dans un scénario de validation du régime alimentaire, un modèle élaboré à partir d'une connaissance limitée du régime alimentaire de l'Éperlan arc-en-ciel (*Osmerus mordax*) a permis l'estimation adéquate de $\delta^{13}C$ et de $\delta^{15}N$ par rapport à un modèle nul, mais n'a pu déterminer correctement la consommation des proies. Dans un second scénario dans lequel des touladis se sont, pendant une courte période, alimentés par cannibalisme d'estivaux de touladis ensemencés, la possibilité de détection de l'augmentation de $\delta^{15}N$ reliée au cannibalisme dépendait de la taille du prédateur, de la durée du cannibalisme et de la taille de l'échantillon. Dans un dernier scénario où la variation isotopique saisonnière se produisait à la base du réseau alimentaire, la variation qui se propageait aux niveaux trophiques supérieurs dépendait de la taille du consommateur et de son régime alimentaire. C'est dans l'examen des multiples combinaisons de régimes alimentaires qui produisent les rapports d'isotopes stables observés que notre approche s'avère le plus utile; on peut alors identifier les régimes alimentaires les plus appropriés à l'aide de tests sur le terrain et d'autres observations.

Introduction

Stable isotope analysis is widely used to establish trophic linkages in food webs (e.g., Kling et al. 1992; Hecky and Hesslein 1995; Cabana and Rasmussen 1996). Trophic transfer causes fractionation of some isotopes in consumers relative to their diets, which allows inference of a consumer's diet by comparing its isotopic ratios with those of other species in its food web. Such inferences can be tested by complementary, periodic examinations of stomach contents. This method is most powerful when several isotope ratios are studied simultaneously, because the resolution of potential food web linkages is greatly improved (Peterson et al. 1985). For example, the stable isotope ratios of carbon and

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C.J. Harvey,[1,2] P.C. Hanson, T.E. Essington,[3] and J.F. Kitchell. Center for Limnology, University of Wisconsin, Madison, WI 53706, U.S.A.
P.B. Brown. Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, U.S.A.

[1]Present address: Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA 98112, U.S.A.
[2]Corresponding author (e-mail: chris.harvey@noaa.gov).
[3]Present address: Marine Science Research Center, State University of New York, Stony Brook, NY 11794, U.S.A.

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nitrogen, $\delta^{13}C$ and $\delta^{15}N$ (notation of Peterson and Fry 1987), are often simultaneously evaluated. In theory, $\delta^{15}N$ increases by ~3.4‰ with each trophic transfer (Minigawa and Wada 1984) and thus is an indicator of trophic level. In contrast, $\delta^{13}C$ increases <1‰ with each trophic transfer (Michener and Schell 1994), and thus an organism retains the approximate $\delta^{13}C$ signature of its ultimate source of primary production.

Static mixing models are often used to estimate the importance of different prey items or production bases to a consumer (Phillips and Gregg 2001). The proportions of different prey items that a consumer assimilates can be estimated by simultaneously solving several linear mixing models and a mass balance equation, for example,

$$(1) \quad \delta^{15}N_{predator} = ax + by + cz + \text{fractionation}$$

$$(2) \quad \delta^{13}C_{predator} = au + bv + cw + \text{fractionation}$$

$$(3) \quad 1 = a + b + c \ (0 \leq a, b, c \leq 1)$$

where a, b, and c are the proportions of three prey assimilated in the predator's tissues; x, y, and z are their respective $\delta^{15}N$ values; and u, v, and w are their respective $\delta^{13}C$ values. Fractionation estimates are subject to uncertainty, because fractionation may vary depending on a consumer's nutritional status, diet quality, assimilation efficiency, size, age, and dietary ontogeny (Minigawa and Wada 1984; Hobson and Welch 1995; Ben-David and Schell 2001).

The mixing model method implicitly assumes that a consumer is in isotopic equilibrium with its diet. This assumption is problematic, because isotopic equilibration of a consumer is gradual following a diet switch. Hesslein et al. (1993) found that broad whitefish (*Coregonus nasus*) that grew three- to nine-fold over a 1-year period following a diet switch were not yet isotopically equilibrated with their new diets. Vander Zanden et al. (1998) found that larval smallmouth bass (*Micropterus dolomieu*) approached isotopic equilibrium with a new diet following a three- to four-fold increase in mass. This equilibration occurred far more quickly (~20 days) than in the Hesslein et al. (1993) experiment, probably because the mass-specific growth rate of the larval bass was greater than that of the whitefish. Growth accounted for most of the variation in stable isotope ratios in both of the above studies. These studies show that a consumer's stable isotope ratios depend on its dietary ontogeny and growth rate. A consumer is not necessarily in isotopic equilibrium with its current diet because of the isotopic "memory" of previously assimilated prey items. Thus, the equilibrium assumption of static mixing models ignores the temporally dynamic nature of stable isotope ratios. Such models may therefore misrepresent long-term diets of consumers and bias fractionation estimates.

The general purpose of this paper is to show how changes in fish growth, caused by dietary ontogeny and bioenergetics principles, influence stable isotope ratios in fishes. We present a new approach to modeling stable isotopes by linking isotope ratios to mechanistic models of fish growth based on bioenergetics principles. This approach allows prediction of temporal isotope dynamics that occur as fish diets and growth rates change and thus avoids equilibrium assumptions. It also includes variation in several parameters and thus generates distributions of predator isotope ratios rather than point estimates. We present results from a diet-switch experiment and compare the empirical data to predictions of the model. We then demonstrate the model's utility with three heuristic exercises.

Methods

Model description

Our model is an extension of Fish Bioenergetics 3.0 (Hanson et al. 1997), a dynamic fish energy budget that follows the thermodynamic principle that energy input (consumption) and outputs (respiration, waste, growth) must balance (Kitchell et al. 1977). Bioenergetics models project the growth of a fish through time, accounting for temperature, fish size, and diet quality. Bioenergetics parameters exist for many species (Hanson et al. 1997), and the user provides temporally explicit data on diet, temperature, energy densities, and predator starting and ending weights. From these values, the model calculates daily fish growth and prey consumption.

We incorporated temporal dynamics of stable isotopes into the bioenergetics model with an equation, adapted from Hesslein et al. (1993), that relates stable isotope ratios to growth and tissue turnover. The stable isotope ratio on day t (δ_t) was calculated as

$$(4) \quad \delta_t = [(\delta_{t-1} \cdot B_{t-1} + (B_t - B_{t-1}) \cdot \delta_{eq})/B_t] - [m(\delta_{t-1} - \delta_{eq})]$$

where δ_{t-1} is the stable isotope ratio on day $t - 1$, B is wet biomass, δ_{eq} is the fish's theoretical δ when at isotopic equilibrium with its current diet, and m is a metabolic turnover constant (see Diet-switch experiment). The first bracketed term reflects growth dilution. The second term represents isotopic change resulting from daily tissue turnover, based on a discrete solution for the differential equation $d\delta/dt = -m(\delta_{t-1} - \delta_{eq})$. These equations represent δ values in dorsal white muscle tissue, the tissue used in most isotopic studies of fishes (Pinnegar and Polunin 1999).

We constrained the model so that a loss of fish mass (resulting from starvation, for example) does not result in changes in isotopic signatures in the growth-dilution portion of eq. 4. This follows the findings of Doucett et al. (1999), who saw no change in the white muscle $\delta^{15}N$ of fasting Atlantic salmon (*Salmo salar*) during spawning migrations. Doucett et al. (1999) attributed minor increases in white muscle $\delta^{13}C$ to extracellular lipid depletion rather than to enrichment of the muscle tissue. Thus, $\delta^{13}C$ values in this model should be corrected for lipids, which are depleted in ^{13}C relative to most other tissues (DeNiro and Epstein 1978).

The model has several sources of error: variation in δ values of predator and prey, variation in diet composition, and variation in fractionation. Natural variation in prey isotope ratios and fractionation is incorporated by drawing δ values and fractionation estimates from normal distributions with means and standard deviations derived from empirical data or literature values. The amounts of each prey item in the diet are randomly drawn from empirically derived normal distributions and then rescaled so that they are proportions that sum to 1. Finally, the model draws initial ($t = 0$) consumer δ values from an empirically derived normal distribution. The model is then run repeatedly to simulate dynamics of stable isotope ratios over a given time period and to gen-

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erate distributions of δ values for the consumer. In subsequent sections, we describe the use of this model in empirical and hypothetical scenarios.

Diet-switch experiment

To validate the model, we conducted an experiment to evaluate stable isotope dynamics following a sudden shift in diet composition. Juvenile lake trout (*Salvelinus namaycush*; mean weight ~25 g) were held in two 450-L flow-through tanks at densities of 60 fish per tank. The tanks were continuously supplied with fresh, oxygenated, 10.6°C well water. Fish were fed twice daily (2% body weight·day^{-1}) with the pellet diet upon which they were reared in a hatchery (Table 1). Fish acclimated to these conditions for 10 weeks.

After acclimation, fish were anesthetized (MS-222) and weighed. Five fish from each tank were sacrificed and frozen. All other fish were given weight-specific fin-clip combinations (e.g., all 43-g fish were given a clip on the lower caudal lobe and the right pelvic fin) and revived. Fish in one tank remained on the control diet, and fish in the other tank received a treatment diet enriched in both ^{15}N and ^{13}C by ~3‰ over the control diet (Table 1). Three to five fish per tank were sacrificed on subsequent dates (days 5, 12, 19, 26, 33, 47, 70, 90, 111, and 136 since the diet switch). Each fish (with gut contents removed via dissection) was weighed, and its initial weight was determined from its fin clips. Four fish from the treatment group had unreadable fin clips and were discarded. Sacrificed fish were frozen for stable isotope analysis. Subsamples of both diets were frozen for analysis at 3-week intervals.

Dorsal white muscle was dissected from frozen fish. Muscle and diet samples were dried at 60°C to constant mass and ground with a mortar and pestle. Samples were analyzed for δ^{13}C, δ^{15}N, percent carbon (%C), and percent nitrogen (%N) on a Europa 20/20 continuous flow mass spectrometer in series with a Carlo Erba CHN analyzer (University of Wisconsin–Madison Horticulture Department). Precision for replicated stable isotope samples was ±0.2‰ for both elements. We extracted lipids from a subsample (n = 37) of lake trout and a sample of both diets. Extraction occurred in boiling petroleum ether for 4–6 h in a Goldfisch extraction apparatus, using AACC (1995) methods. Extracted samples were dried and reanalyzed for δ^{13}C and δ^{15}N. Following inverse transformation of all variables, multiple regression showed that δ^{13}C and %N prior to extraction were the best predictors of lipid-corrected δ^{13}C (n = 39; r^2 = 0.94; p < 0.0001). We used this regression to correct δ^{13}C values of all other samples against lipid ^{13}C depletion bias (DeNiro and Epstein 1978).

We compared observed stable isotope dynamics with model simulations, using lake trout bioenergetics parameters of Stewart et al. (1983). Simulated temperature equaled 10.6°C, starting weight equaled the mean of all fish prior to the diet switch, and final weight equaled the mean of treatment fish sampled on day 136. Starting δ^{13}C and δ^{15}N were drawn from distributions described by the means and standard deviations (SD) of the fish sacrificed on day 1, and δ^{13}C and δ^{15}N of the diets were similarly drawn randomly from distributions based on diet samples. The δ_{eq} values equaled the δ values of the diets plus the assumed mean fractionations, which for this exercise equaled the isotopic differences between mean control fish and mean control diet, with standard deviations derived from the literature (±1.1‰ for ^{13}C (DeNiro and Epstein 1978); ±0.3‰ for ^{15}N (Vander Zanden et al. 1997)). Following the methods of Hesslein et al. (1993), we estimated the turnover constant (m in eq. 4) using individual fish growth data. Model simulations lasted 136 days. We ran 1000 iterations of the model and compared the simulated δ^{13}C and δ^{15}N values with those observed in the experiment.

Model applications

The model was used to examine temporal trends in stable isotope ratios of fishes in three hypothetical applications, described below. In the applications, the mean fractionations (±SD) for ^{15}N and ^{13}C were set at 3.4‰ (±0.3) and 0.9‰ (±1.1), respectively (Vander Zanden et al. 1997; M.J. Vander Zanden, Center for Limnology, University of Wisconsin–Madison, personal communication), although we recognize that the magnitudes of fractionations are still a topic of debate (e.g., Adams and Sterner 2000). We set m at 0.0005. Simulations were run 1000 times.

The first application was a diet reconstruction of a fish, age-1 Lake Superior rainbow smelt (*Osmerus mordax*), with the goal of determining if stable isotopes can be used to validate fish diets. We created two models: a null model that represented actual smelt diets, and a model based on limited diet information representative of a seasonal sampling regime. We used smelt bioenergetics parameters of Lantry and Stewart (1993) and smelt thermal regimes, diets, and prey energy densities from Johnson et al. (1998). We assumed the diet in Fig. 1 to be the "true" smelt diet, with standard deviations of prey proportions arbitrarily set at 0.4 × (proportion). Mean δ^{15}N and δ^{13}C and standard deviations for smelt and their prey are from Harvey and Kitchell (2000). We ran 1-year simulations (day 1 = June 1) with the "true" diet to estimate consumption and δ values for the null model. We then ran "uninformed" simulations in which we assumed diet estimates were only available for four dates over the 1-year simulation. We used the diet proportions for days 1, 62, 123, and 365 (June 1, August 1, October 1, and June 1) and linearly interpolated diet proportions between those dates. We compared values from the "uninformed" and "true" models and also compared the estimated δ values on the four dates above with values estimated by a static mixing model, given the diet composition on those dates.

As an extension of the first application, we ran a second application to determine the effectiveness of stable isotope analysis in detecting short-term "pulses" of predation on a food source with a distinct isotopic signal. As implied in the first exercise, field sampling of fish for stomach content or stable isotope analysis is not continuous, but rather incremental, and sampling increments are often separated by several months. Important foraging events may occur between sampling increments and not be evident in gut content samples. We simulated a situation in which native lake trout in Lake Superior were preying upon recently released hatchery lake trout fingerlings, a situation hypothesized by Hansen et al. (1996) as a source of mortality for stocked lake trout. We used lake trout bioenergetics parameters of Stewart et al. (1983), temperatures, diets, energy densities, starting weights, and ending weights described by Negus (1995), and

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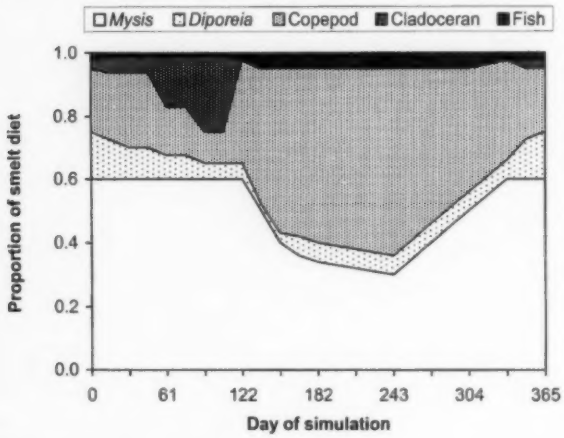
Table 1. Composition and stable isotope values of the control (hatchery) and treatment diets used in the diet-switch experiment.

Ingredient	Control diet	Treatment diet
Fish meal	30% (menhaden, herring, anchovy)	45.5% (anchovy)
Wheat	22.0%	0.0%
Soybean meal	25.0%	17.8%
Corn grain	0.0%	18.0%
Blood meal	10.0%	11.4%
Fish oil	10.0%	6.3%
Pellet binder, vitamins, minerals	3.0%	1.0%
$\delta^{13}C$ (mean ± SD)	–20.21‰ ± 0.24	–17.94‰ ± 0.21
$\delta^{15}N$ (mean ± SD)	6.41‰ ± 0.42	9.51‰ ± 0.47

Note: SD, standard deviation.

Fig. 1. Diet for age-1 rainbow smelt in western Lake Superior (data from Johnson et al. 1998). Diet proportions in this plot were used as the "true" diet in model Application 1 (day 1 = June 1).



$\delta^{15}N$ and standard deviations measured by Harvey and Kitchell (2000). We simulated $\delta^{15}N$ dynamics for age-4 and age-7 Lake Superior lake trout for 120-day simulations. We simulated lake trout that consumed stocked lake trout for 0, 5, 15, or 30 days before reverting to the reference diet reported by Negus (1995). When feeding on stocked trout, adult trout fed at the maximum physiological rate. Stocked lake trout $\delta^{15}N$ was set to 10.39‰ ± 0.37, based on experimental results in this study. We conducted a power analysis to determine the sample sizes necessary to detect a significant isotopic difference between cannibals and noncannibals at the end of the simulated time period.

The third application was to determine how temporal variation in δ values at the base of a food web affected δ values at higher trophic levels. We simulated a zooplankton–planktivore–piscivore food chain. Zooplankton $\delta^{15}N$ increased from 1‰ on May 1 to 12‰ on September 1 and returned to 1‰ by January 1, with a constant SD of ±1.0; the zooplankton $\delta^{15}N$ increase is similar to that observed in Lake Ontario by Kiriluk et al. (1995). Zooplankton were eaten by alewife (*Alosa pseudoharengus*), which were eaten by lake trout. Simulations ran 1 year (day 1 = July 1). We modeled alewife $\delta^{15}N$ using bioenergetics parameters, temperatures, growth data, and seasonal energy densities from Stewart and Binkowski (1986). Three alewife cohorts (young-of-year (YOY), age-1 juveniles, and age-2 adults) were modeled. Alewife began simulations in isotopic equilibrium with day-1 zooplankton (initial alewife $\delta^{15}N$ = 9.9‰ ± 0.6). We used lake trout bioenergetics parameters from Stewart et al. (1983) and temperature and growth data from Madenjian et al. (1998) to model $\delta^{15}N$ for age-2 and age-8 lake trout in nearshore Lake Michigan. Lake trout began in isotopic equilibrium with alewife (initial lake trout $\delta^{15}N$ = 13.3‰ ± 0.6). Lake trout cohorts were modeled with three diets: 100% YOY alewife, 100% juvenile alewife, or 100% adult alewife.

Results

Diet experiment and model comparison

Over the course of the experiment, lake trout increased in mass by three- to six-fold (Fig. 2). Growth patterns in the control and treatment groups were relatively similar until the final sampling increment. Control fish exhibited exponential growth during the experiment, whereas growth in the treatment group decelerated after day 111. Treatment fish sacrificed on day 136 had experienced identical growth as fish sacrificed on day 111. Fin clips were unreadable for four treatment fish, all sacrificed on one of the final three sampling dates, potentially affecting the estimate of the daily ration and causing underfeeding in the treatment tank.

Stable isotope values of diet samples (Table 1) did not vary significantly with time during the experiment (linear regressions; n = 8; p values > 0.1). Initial $\delta^{15}N$ and $\delta^{13}C$ for control fish, 10.23‰ (±0.47) and –18.52‰ (±0.16), respectively, did not differ from those of treatment fish, 10.27‰ (±0.19) and –18.68‰ (±0.08), respectively. Comparisons of the initial isotope ratios of the fish and the control diet indicated fractionations of 3.89‰ for $\delta^{15}N$ and 1.55‰ for $\delta^{13}C$. Following the diet switch on day 1 of the experiment, δ values of the treatment group diverged from those of the control group and approached equilibrium with the treatment diet (Figs. 3*a*, 3*b*).

To validate the model, we compared observed and simulated lake trout stable isotope ratios. We estimated the turnover constant m by fitting values for individual treatment group fish predicted by eq. 4 against observed values and adjusted m to minimize the residual sum of squares (Hesslein et al. 1993). For both $\delta^{15}N$ and $\delta^{13}C$, m was estimated as 0.0005. We then used the full model to predict δ values for lake trout in the treatment group. Initial and final

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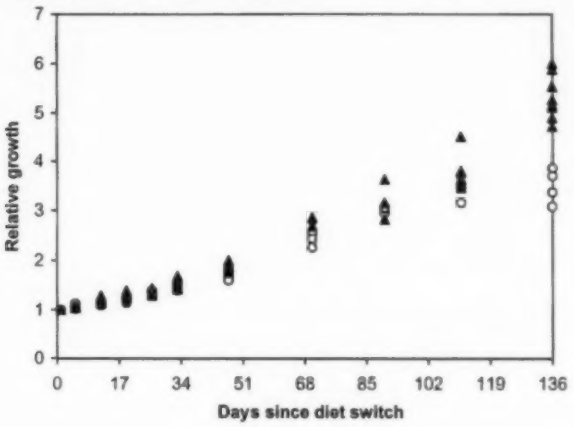
Fig. 2. Observed relative growth of juvenile lake trout (▲, control fish; ○, treatment fish) in the diet-switch experiment. Relative growth, calculated for each individual, equals final weight divided by initial weight.



weights for model fish were 55 g and 196 g, respectively, and the model closely matched observed weights over time (Kolmogorov–Smirnov goodness-of-fit test, $p > 0.2$). Predictions and observations were similar for $\delta^{15}N$ (Fig. 3*c*). Initial simulations of $\delta^{13}C$ dynamics strongly overestimated final $\delta^{13}C$ values. Thus, fractionation for ^{13}C was lowered to 0.9‰. Despite this adjustment, the model did not accurately predict $\delta^{13}C$ between days 5 and 47 (Fig. 3*d*). Error around the mean prediction increased through time, probably because of the large standard deviation of ^{13}C fractionation.

Model application 1: diet validation

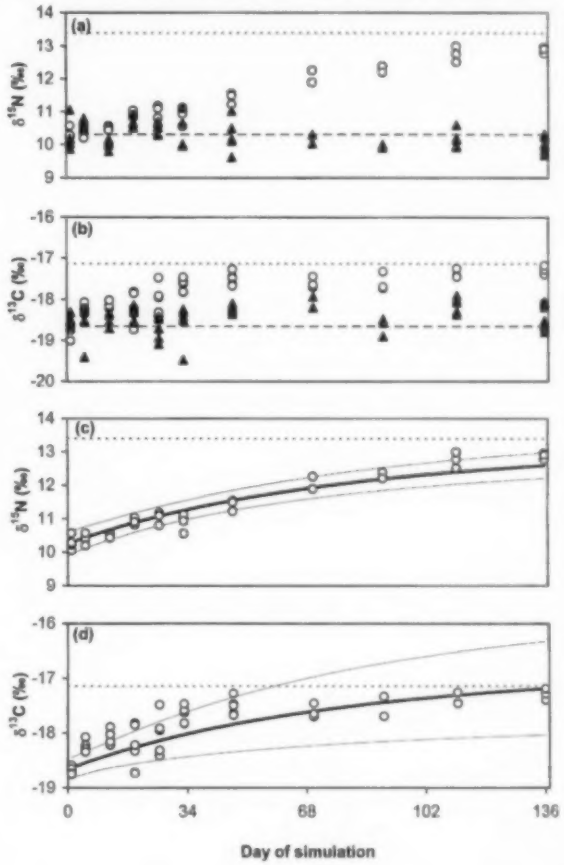
The first model exercise explored whether limited gut content and stable isotope data could produce a valid estimate of the actual diet of a fish. "True" and "uninformed" models of age-1 Lake Superior rainbow smelt produced nearly identical growth trajectories (Fig. 4*a*) and stable isotope dynamics (Figs. 4*b*, 4*c*). Both models predicted that $\delta^{15}N$ would plateau between 7.2‰ and 7.5‰ and that $\delta^{13}C$ would remain stable. Variation decreased over the course of all model runs. There were no differences between distributions of isotope ratios on days 62, 123, or 365 of the two bioenergetics simulations (*t* tests, all $p > 0.05$). However, static mixing models overestimated $\delta^{15}N$ compared with the bioenergetics models (Fig. 4*b*). The two methods produced similar estimates for $\delta^{13}C$ (Fig. 4*c*).

Although the two models produced similar isotopic results, which suggests that the limited diet sampling regime reasonably depicted actual smelt diets, they generated very different patterns of consumption on prey populations. The "uninformed" model slightly overestimated the total consumption of the major prey item, *Mysis*, but greatly overestimated total consumption of *Diporeia* and underestimated consumption of cladocerans, copepods, and other fishes, compared with the "true" model (Table 2).

Model application 2: influence of feeding pulses

The effect of a feeding pulse on growth and stable isotope ratios of lake trout depended on both predator size and the duration of the feeding pulse. Growth was most rapid among lake trout that were cannibalistic for 30 days (Figs. 5*a*, 5*b*). Relative weight gain was greater in age-4 lake trout than in age-7 lake trout. Lake trout that cannibalized hatchery lake trout fingerlings for 5 days, 15 days, and 30 days all showed a change in $\delta^{15}N$ that remained distinguishable from the reference population until the end of the simulation (Figs. 5*c*, 5*d*; Table 3). Significant results, however, were based on comparisons of 1000 individuals, which is unrealistic for an actual field study. Power analysis on each *t* test revealed that up to 652 samples were needed on day 120 to correctly distinguish cannibalistic lake trout from reference trout 95% of the time, depending on predator size and cannibalism duration (Table 3).

Fig. 3. Stable isotope ratios for juvenile lake trout in control (▲) and treatment (○) groups in the diet-switch experiment. (*a*) $\delta^{15}N$ and (*b*) $\delta^{13}C$ in control and treatment fish; broken lines indicate isotopic equilibrium values relative to the control (– – –) and treatment (- - -) diets. (*c*) $\delta^{15}N$ and (*d*) $\delta^{13}C$ in treatment fish compared with model predictions; the heavy line is the mean model prediction and the lighter lines represent ±1 standard deviation.



Model application 3: variation in isotopic baselines

Responses of higher trophic levels to isotopic variation at the base of a food web were dependent on consumer size, diet, and trophic level. Growth of alewife cohorts was oscil-

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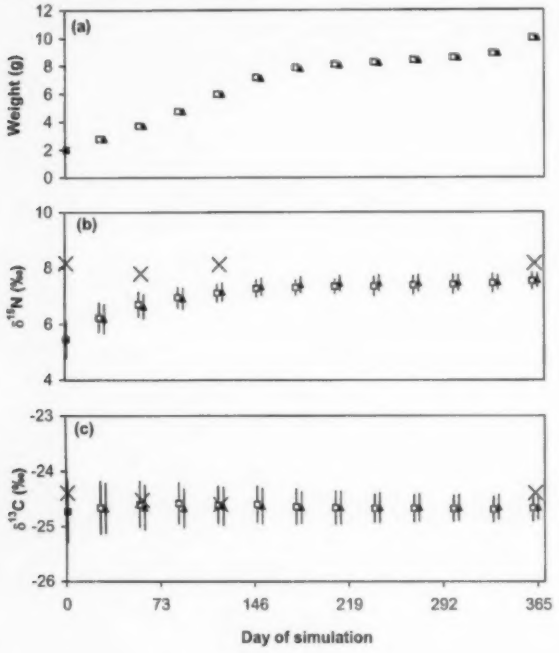
Fig. 4. Simulated weight and stable isotope ratios of rainbow smelt in Application 1 (day 1 = June 1). The "true" diet is from Fig. 1; the "uninformed" diet was known only on days 1, 60, 120, and 365 and was interpolated for all other days. (*a*) Weights of the two smelt populations; (*b*) mean $\delta^{15}N$ (± standard deviation (SD)) and (*c*) mean $\delta^{13}C$ (± SD), with predictions by a mixing model. □, "true" diet population; ▲, "uninformed" diet population; ×, mixing model prediction.



latory and differed according to alewife size (Fig. 6*a*). YOY alewife $\delta^{15}N$ tracked changes in zooplankton $\delta^{15}N$ with only a brief lag (Fig. 6*b*). Age-1 and adult alewife also tracked changes in zooplankton $\delta^{15}N$, although the magnitude and rate of isotopic change was lower in both cases. When zooplankton $\delta^{15}N$ peaked (day 61), mean YOY alewife $\delta^{15}N$ was ~2.5‰ greater than adult alewife $\delta^{15}N$, which was the greatest difference between alewife isotope ratios observed in the simulation. Age-1 alewife experienced a less protracted period of decreasing $\delta^{15}N$ because they began losing weight on day 122, 30 to 45 days earlier than the other cohorts. Lake trout growth depended on their initial size and their prey (Figs. 7*a*, 7*b*). Age-2 lake trout $\delta^{15}N$ responded more than that of age-8 lake trout with the same diet (Figs. 7*c*, 7*d*) such that the age-2 trout appeared to occupy a higher trophic level. Isotopic change for both lake trout age classes was smallest when preying on adult alewife. The standard deviations of alewife and lake trout $\delta^{15}N$ consistently decreased over the course of the simulations (Figs. 6*b*, 7*c*, 7*d*). Overall, trophic level was inversely related to the range of $\delta^{15}N$ values. Zooplankton $\delta^{15}N$ ranged from 1‰ to 12‰, whereas the greatest range for alewife was for YOY (9.9‰ to 13.1‰), and the greatest range among lake trout was for age-2 lake trout eating YOY alewife (13.3‰ to 14.8‰).

Discussion

Food web studies that employ stable isotope analysis must address the dynamic nature of stable isotope ratios. In the laboratory study and applications, consumers were rarely in isotopic equilibrium with their diets, and thus mixing models would have inaccurately predicted stable isotope ratios. Other studies have examined stable isotope ratio dynamics; Hesslein et al. (1993) and Vander Zanden et al. (1998) observed changes in stable isotope ratios of fishes following diet switches. Both studies featured models of isotopic change as a function of growth, although their models assumed exponential growth. This assumption may not apply to cases where fishes experience pronounced changes in temperature, energy density, or prey quality (e.g., alewife in this study; Stewart and Binkowski 1986). The bioenergetics approach explicitly accounts for temperature, energy densities, and size-specific physiological rates (Kitchell et al. 1977) and thus may produce more realistic trajectories of isotopic change.

The results of the applications demonstrate several concepts that are critical in applying stable isotope analyses to field studies: (*i*) stable isotope ratios are a function not only of diet and fractionation, as depicted in a mixing model, but also of consumer growth patterns; (*ii*) stable isotope ratios may give misleading results, even when empirical and model results are very similar; (*iii*) seasonal isotopic variation at the base of a food web is propagated to higher levels of a food web with decreasing amplitude and may confound interpretation of stable isotope data; and (*iv*) sufficient sample size is very important if one wishes to use stable isotope ratios to detect short-term diet switches or pulse feeding events. These ideas are developed below.

Growth rates explain most of the variation in a consumer's stable isotope ratios (Hesslein et al. 1993; Vander Zanden et al. 1998). Fish growth is a function of temperature and prey quality, and growth rates further depend on fish size because mass-specific consumption and respiration rates generally decrease as fish size increases (Kitchell et al. 1977). Temperature, prey quality, and fish size interact such that within a species, the optimal growth temperature may change depending on fish size, ration size, and prey energy density (Kitchell 1983). For example, in Application 3, YOY and adult alewife continued to grow for several weeks after age-1 alewife began to lose weight as a function of temperature (Stewart and Binkowski 1986). This caused the $\delta^{15}N$ of YOY and, to a lesser extent, adult alewife to decrease as zooplankton $\delta^{15}N$ decreased, whereas age-1 alewife $\delta^{15}N$ remained stable over the same time period. Thus, YOY and adult alewife $\delta^{15}N$ were influenced by fish size, and age-1 alewife $\delta^{15}N$ was affected by suboptimal temperatures that caused weight loss.

Our results suggest that stable isotopes are not always a reliable means of diet validation. In Application 1, diet samples collected from four dates in 1 year resulted in highly inaccurate consumption estimates, even though the δ values were similar to "true" values. The discrepancy was caused by the sampling schedule: sampling on October 1 (day 123 of the simulation) occurred before smelt shifted to a primarily copepod diet, and the subsequent sampling on June 1

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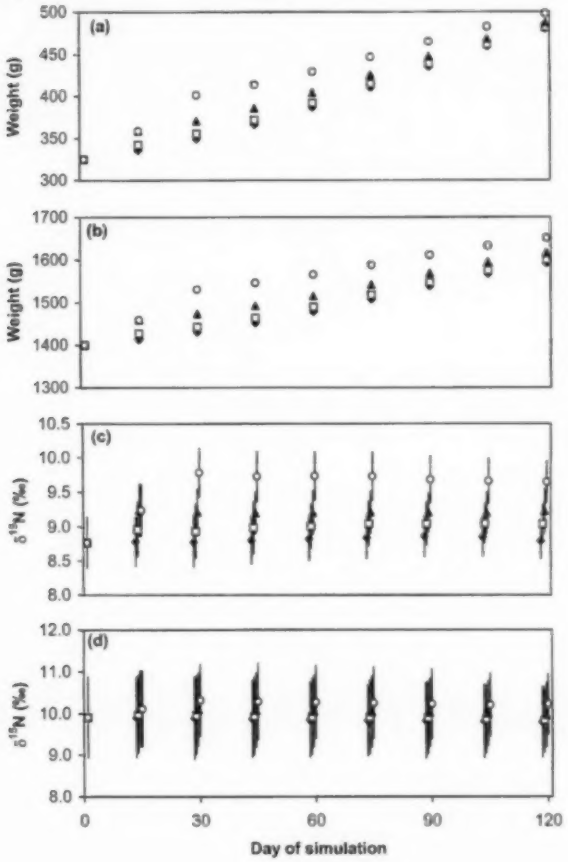
Table 2. Total consumption estimates and ratios as predicted by the "true" and "uninformed" models of age-1 Lake Superior rainbow smelt.

	Total consumption by smelt (g)				
Model	Cladocerans	Copepods	*Mysis*	*Diporeia*	Fish
Uninformed	1.94	12.84	31.25	4.86	1.19
True	2.60	18.58	26.79	3.69	1.76
Uninformed:True	0.75	0.69	1.17	1.32	0.68

Fig. 5. Simulated weight and $\delta^{15}N$ of adult lake trout in Application 2 (day 1 = June 1). Adult trout fed on hatchery lake trout for the initial 0, 5, 15, or 30 days (◆, □, ▲, and ○, respectively) before reverting to a reference diet with no cannibalism. (*a* and *b*) Weights for age-4 and age-7 adult lake trout, respectively; (*c* and *d*) mean $\delta^{15}N$ (± standard deviation) for age-4 and age-7 adult lake trout, respectively.



(day 365) took place after the smelt reverted to a primarily macroinvertebrate diet. This sampling schedule may be pragmatic because of poor sampling conditions in the late fall, winter, and early spring in some aquatic systems, but a consequence may be that diet estimates from gut contents are unrealistic, despite seemingly accurate δ values generated by accompanying models. This conclusion is not meant to discourage the joint use of stable isotope analysis and gut content analysis in food web studies but rather to encourage careful examination of the results of both analyses because of potential unobserved variability in fish diets (see also Persson and Hansson 1999). For example, one might use the model to examine several possible diets during long periods in which no sampling occurs. In that way, diets that might result in significant isotopic deviation can be identified, uncertainty around diet proportion and consumption estimates can be quantified or minimized, and testable hypotheses can help guide future sampling plans.

Seasonal variation in δ values at lower trophic levels has been observed previously (e.g., Yoshioka et al. 1994; Kiriluk et al. 1995) and is probably caused by biogeochemical factors that affect stable isotope ratios of primary producers. For example, phytoplankton $\delta^{13}C$ is dependent on the available forms of dissolved inorganic carbon, determined in part by temperature; on the metabolic pathway(s) used to fix carbon; and on phytoplankton growth rates, which are further influenced by light and trace nutrient concentrations (Michener and Schell 1994). Phytoplankton $\delta^{15}N$ is similarly dependent on the composition of dissolved inorganic nitrogen, which varies based on rates of biotic nitrogen recycling in surface waters and nitrogen influx from deep waters or external sources (Michener and Schell 1994). Our approach demonstrates that fluctuating δ values at the base of a food web are dampened at higher trophic levels, as postulated by Wada et al. (1987). The zooplankton–alewife–lake trout food chain in Application 3 depicts this tendency, as the seasonal fluctuation in zooplankton $\delta^{15}N$ was progressively reduced in alewife and lake trout $\delta^{15}N$, especially large lake trout that were eating large alewife. Such fluctuations may be further dampened by species at intermediate trophic positions. For example, in the Great Lakes, macroinvertebrates such as *Mysis relicta* consume zooplankton and are themselves eaten by alewife, which are then eaten by lake trout (Kiriluk et al. 1995). Seasonal fluctuations are likely greatest in organisms with high growth and turnover rates, such as small organisms at the base of a food web (Cabana and Rasmussen 1996). In fishes, the greatest mass-specific growth occurs in small individuals. Rapid growth coupled with a large seasonal increase in δ values of lower trophic levels may cause small fish to be isotopically enriched relative to large conspecifics, as in Application 3. This may create the impression that smaller individuals occupy a trophic position equal to or higher than larger individuals, which conflicts with basic principles of trophic ontogeny. Thus, our model may help explain cases in which young individuals have $\delta^{15}N$ levels equal to or greater than older conspecifics (e.g., Spies et al. 1989; Kiriluk et al. 1995).

The last key concept is that sample size is important if stable isotopes are used to detect brief feeding episodes not

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Table 3. Comparisons of $\delta^{15}N$ of lake trout in Application 2.

Period of cannibalism	Mean $\delta^{15}N$ on day 120, ‰ (±SD)	*t* value	*p* value	Sample size required
Age-4 lake trout				
None (null model)	8.85 (0.26)	—	—	—
Days 1–5	9.04 (0.27)	15.05	<0.0001	17
Days 1–15	9.21 (0.29)	28.99	<0.0001	5
Days 1–30	9.63 (0.31)	59.93	<0.0001	2
Age-7 lake trout				
None (null model)	9.75 (0.83)	—	—	—
Days 1–5	9.83 (0.79)	2.35	0.02	652
Days 1–15	9.96 (0.78)	5.80	<0.0001	107
Days 1–30	10.22 (0.72)	13.42	<0.0001	20

Note: Fish in the null models ate the diets presented in Negus (1995). Fish in other models cannibalized hatchery lake trout for the time indicated. *t* tests were used to compare null vs. cannibalistic models, and power analysis was used to determine the sample size required to correctly identify significant differences 95% of the time. SD, standard deviation.

Fig. 6. Simulated weight and $\delta^{15}N$ of young-of-year (□), age-1 (▲), and adult (●) alewife in Application 3 (day 1 = July 1): (*a*) weights of alewife cohorts; (*b*) mean $\delta^{15}N$ (± standard deviation) of zooplankton (×) and alewife cohorts.

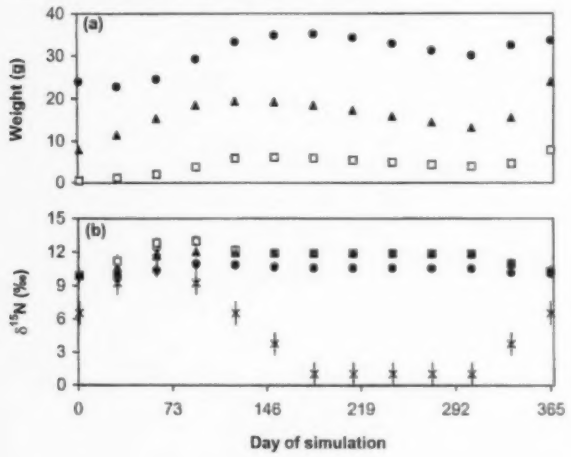
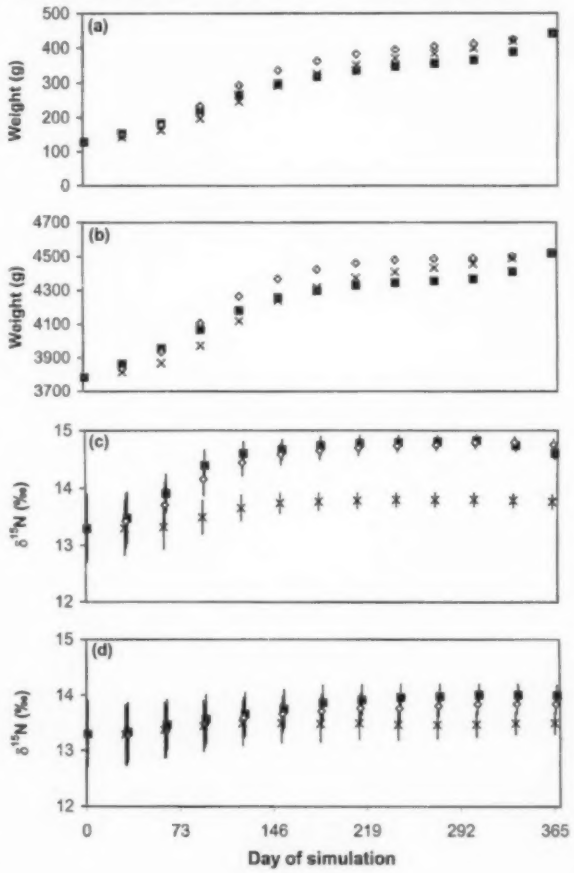


Fig. 7. Simulated weight and $\delta^{15}N$ of age-2 and age-8 lake trout feeding exclusively on young-of-year (■), age-1 (◇), or adult (×) alewife in Application 3 (day 1 = July 1): (*a*) weights of age-2 lake trout; (*b*) weights of age-8 lake trout; (*c*) $\delta^{15}N$ (± standard deviation (SD)) of age-2 lake trout; (*d*) $\delta^{15}N$ (± SD) of age-8 lake trout.



readily observed through stomach analysis. In Application 2, predator growth rates were important determinants of the isotopic response to the prey switch and thus influenced the sample size needed to detect the prey switch. Similar cases may involve predators feeding on small fishes migrating through the predators' habitat or seasonal predation on eggs or posthatch larvae. Prior to sampling, power analysis should be conducted to determine the sample size required to detect anticipated isotopic differences resulting from feeding pulses. Stable isotope studies of food webs often involve 5–8 samples of a species per site (e.g., Harvey and Kitchell 2000), but this tradition should be abandoned if it creates a strong risk of Type II errors for studies such as that simulated in Application 2 (see also Owens 1987). Furthermore, certain conditions must exist for such an event to be detected. First, prey in the feeding pulse must be isotopically distinct from the predator's normal diet. Second, predators must grow substantially during the pulse, which was the case with age-4 lake trout but less so with age-7 lake trout in

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Application 2. These two conditions may explain why smelt in Application 1 did not show evidence of a diet switch during winter. Third, the initial SD of the predator's δ values cannot be too large. For example, in Application 2, the relatively large initial SD for age-7 lake trout $\delta^{15}N$ greatly increased the sample sizes required to detect isotopic differences. Finally, sample size determinations must be hypothesis driven, not just efforts to attain significant results. Power analyses and sampling efforts should be based upon reasonable expectation that an event, such as a feeding pulse, is occurring. Otherwise, the risk of Type I error becomes increasingly high.

There are aspects of the model that require further study and refinement. For example, the model accurately predicted $\delta^{15}N$ results in the diet-switch experiment, but its performance was poor for $\delta^{13}C$. The model's poor fit to empirical data may have been related to the initial estimate of mean $\delta^{13}C$ or to individual variation of $\delta^{13}C$ in the treatment group. Or, ^{13}C fractionation may have varied over the course of the experiment. Fractionation may change if the nutritional needs of a consumer or the quality of its diet change (Adams and Sterner 2000). However, C:N ratios were similar between the two lake trout groups and did not change during the experiment. An intriguing use of the model would be simulating cases in which fractionation is the unknown, rather than the final δ value. We could thereby develop hypotheses of how fractionation changes through time within the constraints of mass balance.

Another issue requiring study is our assumption that the proportions of prey assimilated are similar or equal to the proportions of prey found in gut contents. In other words, we assumed that consumers assimilated all prey with equal efficiency, an assumption that may not be valid (Ben-David and Schell 2001). This concern is of lesser consequence when prey items are similar (e.g., zooplankton) but increases if prey have very different indigestible components (e.g., crayfish or crabs vs. fishes) and produce different net growth results for the predator (Kitchell 1983). We suggest caution when using this model to validate diets in terms of quantities of prey items eaten, rather than assimilated, particularly in cases with omnivorous fishes.

Accounting for variability remains an issue in stable isotope analysis of food webs (Ben-David and Schell 2001; Phillips and Gregg 2001), and future work with this model must involve careful evaluation of how error is propagated and conserved. While the models presented here accounted for variation and produced distributions of δ values, the behavior of the uncertainty terms did not necessarily mirror observed error in real systems. For example, in Applications 1 and 3, the standard deviations of δ values decreased with time. This decrease is reasonable in theory because variation between individuals in a population should average out through time (Hesslein et al. 1993). Little empirical evidence supports this assertion, however, and some evidence contradicts it. In one study, Kline et al. (1998) found that Dolly Varden char (*Salvelinus malma*) $\delta^{15}N$ and $\delta^{13}C$ variability decreased as the fish grew larger. However, Kline et al. (1998) also found that coregonines in the same system maintained similar variance for both isotopic ratios as they grew. Hobson and Welch (1995) found that $\delta^{15}N$ varied more in large Arctic char (*Salvelinus alpinus*) than in intermediate-sized char. Our model accounts for many sources of variation, primarily by drawing random samples from normal distributions. It is thus possible that fishes and other consumers do not behave as randomly in nature as we have depicted them in this model. Furthermore, the model only develops growth trajectories for fish that grow from a mean starting weight to a mean final weight. Natural variation in growth patterns among individuals adds a further layer of complexity to proper error calculation.

In conclusion, this study illustrates the importance of including growth dynamics in food web studies that employ stable isotopes and further shows that bioenergetics modeling is a framework in which to represent those dynamics. This method offers an alternative to other models, such as linear mixing models described by Phillips and Gregg (2001) or exponential growth models discussed by Hesslein et al. (1993) and Vander Zanden et al. (1998). Mixing models may be the best approach in cases where bioenergetics parameters or growth data are lacking or where the fishes being examined are large, old adults that are more likely to be near isotopic equilibrium with their diets. When such models are used, error estimation is imperative, because these models may be highly inaccurate in predicting the importance of different prey bases (Ben-David and Schell 2001). Alternatively, if fishes exhibit exponential growth, an exponential growth-based model is a practical tool. Our model should prove useful in systems where equilibrium assumptions cannot be met, where exponential growth is not occurring, and where parameters for bioenergetics-style analyses are available. The applications presented here illustrate the model's usefulness in examining the theoretical behavior of stable isotopes in food webs and in evaluating alternative ways in which trophic mechanisms and interactions can produce observed stable isotope values.

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Seawater tolerance and gene expression in two strains of Atlantic salmon smolts

Thomas D. Singer, Koreen M. Clements, Jeffrey W. Semple, Patricia M. Schulte, Jason S. Bystriansky, Bengt Finstad, Ian A. Fleming, and R. Scott McKinley

Abstract: The seawater tolerance of Atlantic salmon (*Salmo salar*) smolts reared under identical hatchery conditions was assessed in two Norwegian strains: AquaGen and Imsa. Plasma ion levels were disrupted in both strains following seawater exposure, but these disruptions were more profound in the AquaGen fish. To investigate the mechanisms underlying these differences, we measured gill Na^+,K^+-adenosine triphosphatase (ATPase) activity and mRNA levels of *Na^+,K^+-ATPase* α-subunit and two isoforms of the cystic fibrosis transmembrane conductance regulator (*CFTR*). Gill Na^+,K^+-ATPase activity rose significantly in both strains following seawater exposure. Both *Na^+,K^+-ATPase* α-subunit and *CFTR I* mRNA levels were significantly elevated for the entire 2-week period following seawater exposure, whereas *CFTR II* levels were transiently elevated during the first 24 h only. There were no differences in enzyme activity or gene expression between strains, with the exception of *CFTR II*, which was significantly lower in the Imsa strain 2 weeks following seawater exposure. This suggests that although changes in mRNA and protein expression for these genes are associated with seawater transfer, they are not the basis of observed physiological differences between strains.

Résumé : Nous avons étudié la tolérance à l'eau de mer de saumoneaux élevés en conditions identiques de pisciculture chez deux lignées norvégiennes du saumon de l'Atlantique (*Salmo salar*), AquaGen et Imsa. Une exposition à l'eau de mer perturbe les concentrations des ions du plasma chez les deux lignées, mais plus fortement chez les poissons AquaGen. Pour étudier les mécanismes qui expliquent ces différences, nous avons mesuré l'activité de la Na^+,K^+-adénosine triphosphatase (ATPase) des branchies et les quantités d'ARNm de la sous-unité α de la *Na^+,K^+-ATPase* et de deux isoformes du régulateur du transport transmembranaire de la fibrose kystique (*CFTR*). L'activité de la Na^+,K^+-ATPase des branchies s'accroît de façon significative chez les deux lignées après une exposition à l'eau de mer. Les quantités d'ARNm augmentent significativement tant chez la sous-unité α de la *Na^+,K^+-ATPase* que chez *CFTR I* pendant les deux semaines complètes qui suivent l'exposition à l'eau de mer; chez *CFTR II*, l'augmentation n'est que temporaire pendant les 24 premières heures. Il n'y a pas de différences dans l'activité enzymatique ou l'expression des gènes entre les deux lignées, excepté chez *CFTR II* de la lignée Imsa dont l'expression est plus faible deux semaines après l'exposition. Il appert donc que, bien que des changements dans les quantités d'ARNm et dans l'expression de protéines de ces gènes accompagnent le transfert à l'eau de mer, ils ne sont pas responsables des différences physiologiques observées entre les deux lignées.

[Traduit par la Rédaction]

Introduction

Normal development of Atlantic salmon (*Salmo salar*) requires growth in both freshwater and seawater. Transfer of fish from freshwater to seawater disturbs osmoregulatory balance and activates osmoregulatory organs. Several studies have demonstrated that rearing conditions affect the ability of salmon smolts to tolerate seawater exposure. For example, hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) have reduced ability to tolerate seawater exposure in comparison with wild counterparts (Shrimpton et al. 1994*a*). Likewise, gill Na^+,K^+-adenosine triphosphatase

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T.D. Singer,[1] K.M. Clements, J.W. Semple, P.M. Schulte,[2] and R.S. McKinley.[3] Waterloo Biotelemetry Institute, Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada.
J.S. Bystriansky. Department of Zoology, University of Guelph, Guelph, ON N1G 2W1 Canada.
B. Finstad. Norwegian Institute for Nature Research, Tungasletta 2, N-7485 Trondheim, Norway.
I.A. Fleming. Coastal Oregon Marine Experiment Station, Oregon State University, Hatfield Marine Science Center, Oregon, U.S.A.

[1]Corresponding author (e-mail: tsinger@sciborg.uwaterloo.ca).
[2]Present address: Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada.
[3]Present address: Faculty of Agricultural Sciences, University of British Columbia, 266B 2357 Main Mall, Vancouver, BC V6T 1Z4, Canada.

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(ATPase) activities, normally elevated during smoltification and indicative of seawater tolerance, were consistently lower in hatchery-reared versus wild salmon from a number of species (*Salmo salar*, Virtanen and Soivio 1985; *Salmo trutta*, Nielsen et al. 1999; *Oncorhynchus kisutch*, Shrimpton et al. 1994*a*). Although these studies have demonstrated the impact of environmental rearing conditions on seawater tolerance, few studies have examined the impact of genetic differences by comparing strains. McGeer et al. (1991) showed that six strains of coho salmon reared under identical environmental conditions had varying degrees of salinity tolerance following seawater challenge, suggesting a genetic basis for the differences. Similarly, a native strain of Atlantic salmon showed enhanced ocean survivorship following river release compared with an AquaGen strain after both had been reared under identical hatchery conditions, suggesting enhanced seawater tolerance of native salmon (Aarestrup et al. 2000). The mechanisms underlying these differences, however, have seldom been examined. Comparisons of strains reared under identical conditions, at all levels of organization including the molecular level, are important in order to determine the basis of these differences.

Regulation of *Na^+,K^+-ATPase* α-subunit mRNA levels during smoltification may be of critical importance, because the expression of this gene increases in the gills of both Atlantic salmon smolts and brown trout (*Salmo trutta*) transferred from freshwater to seawater (Madsen et al. 1995; D'Cotta et al. 2000; Seidelin et al. 2000). However, *Na^+,K^+-ATPase* is only one of several genes that is likely to be important during seawater adaptation. In the euryhaline killifish, mRNA levels of the Cl^- channel *CFTR* (cystic fibrosis transmembrane conductance regulator) have also been shown to increase in the gills following abrupt exposure to seawater, suggesting an important role for this gene in teleost seawater adaptation (Singer et al. 1998). Diffusion of Cl^- through the apically located CFTR Cl^- channels in mammalian airway epithelium and the highly specialized salt-secreting rectal gland of the dogfish shark is considered the rate-limiting step during transepithelial salt transport (Hanrahan et al. 1996). The importance of a CFTR homologue in teleost salt transport has also been demonstrated by electrophysiological measurements of a CFTR-like anion conductance from isolated opercular epithelium from seawater-adapted killifish (Marshall et al. 1995). Immunolocalization studies have been used to localize CFTR to specialized chloride-secreting cells from the gills and opercular epithelium of killifish (Mickle et al. 2000) and the gills of the mudskipper (Wilson et al. 2000). Taken together, these data suggest that changes in the mRNA levels of both the *Na^+,K^+-ATPase* α-subunit and *CFTR* may be critical to surviving seawater exposure in teleosts. The recent cloning of two *CFTR* isoforms from the gills of the Atlantic salmon (Chen et al. 2001) provides a novel opportunity to investigate their role in seawater adaptation of Atlantic salmon smolts and examine differences in gene expression between strains.

This is the first study to compare differences between strains of Atlantic salmon at the level of gene expression. To this end, we have studied the expression of the *Na^+,K^+-ATPase* α-subunit, *CFTR I*, and *CFTR II* following seawater exposure in the gills of Atlantic salmon smolts derived from two distinct Norwegian populations: (*i*) 'AquaGen', derived from eighth-generation farm salmon, and (*ii*) Imsa, derived from first-generation sea-ranched native Imsa River salmon. When compared under identical conditions, these strains differ in behaviour and growth, with the AquaGen strain, selected as a representative farm strain, having been shown to be competitively and reproductively inferior to the Imsa strain, chosen to represent a native strain (Einum and Fleming 1997; Fleming et al. 2000). There are three main objectives to our study: (*i*) to determine if there are differences in the ability of AquaGen and Imsa salmon smolts to adapt to seawater by investigating changes in plasma ion levels and gill Na^+,K^+-ATPase activity; (*ii*) if differences exist, to determine if they are reflected in different gill gene expression patterns of *Na^+,K^+-ATPase* α-subunit, *CFTR I*, and *CFTR II*; and (*iii*) to determine the temporal expression patterns of the two *CFTR* isoforms and *α-Na^+,K^+-ATPase* in the gills of Atlantic salmon smolts following seawater exposure. These studies will provide insight into the extent and basis of physiological differences between strains of Atlantic salmon and increase our understanding of the molecular mechanisms associated with seawater exposure in smolts.

Methods

Fish

Atlantic salmon smolts were derived from two distinct populations. The AquaGen strain originated in Norway's national breeding program at Sunndalsora (AquaGen) and were the offspring (fall 1998) of parents taken as eyed eggs in 1996 to the Norwegian Institute for Nature Research (NINA) station at Ims, southwest Norway (Fleming and Einum 1997). These fish constituted the eighth-generation of the first brood line ("population 1"; Gjedrem et al. 1991) that has undergone intentional selection such that it is now derived principally, if not solely, from the River Namsen, mid-Norway (Gjedrem et al. 1991; Fleming and Einum 1997). Selection was based upon high survival and body weight (Gjedrem et al. 1991). Simultaneously, offspring were derived from spawning first-generation adult sea-ranched salmon collected from the River Imsa in the fall of 1998. The offspring of these salmon were considered native River Imsa stock. The eggs and subsequent juvenile fish from both populations were reared under similar hatchery conditions. Both strains were reared until smolting at age 1+. During this period, the fish were fed ordinary commercial dry diet according to temperature and fish size. At the time of the experiments, the length and weight, respectively, of the fish examined from the two strains were as follows (mean ± standard error): AquaGen, 21.9 ± 0.2 cm, 103.9 ± 3.2 g, n = 88; Imsa, 19.9 ± 0.2 cm, 79.2 ± 2.4 g, n = 89.

Experimental design

All experiments were performed at the NINA Station at Ims, Norway (58°59′N, 5°58′E) during May 2000, coinciding with the annual natural smolt migration period in the River Imsa. Freshwater temperatures from the River Imsa ranged from 14.5°C to 18.7°C, whereas seawater temperatures varied between 6.4°C and 7.9°C during the course of

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Table 1. Daily seawater (SW) and freshwater (FW) temperatures (Temp.) for each exposure time course recorded during study period.

			Exposure time						
Day	Temp. FW (°C)	Temp. SW (°C)	0 h, control	1 h	3 h	8 h	24 h	96 h	2 weeks
1	15.8	6.4						T	T
2	15.7	7.0						R	R
3	16.1	7.3							
4	16.8	7.4		T		T			
5	17.1	7.3		RS		RS			
6	17.9	7.0						S	
7	18.7	6.9	S		T		T		
8	16.5	6.6			RS		R		
9	16.0	6.6					S		
10	15.0	6.7							
11	14.5	7.2							
12	14.7	7.6							
13	14.9	7.9							
14	15.0	7.9							
15	14.6	7.9							
16	14.8	7.9							S

Note: Daily freshwater and seawater temperatures are indicated for exact day of transfer (T) from freshwater holding tank to smaller experimental tank, seawater exposure or freshwater replacement (R), and sampling (S) for each exposure time. Seawater exposure and freshwater replacement occurred concurrently for each exposure time.

the study, with specific temperature ranges for each experiment listed in Table 1. At peak time of smoltification, groups of eight fish from each strain were transferred from two 4000-L freshwater holding tanks to individual 100-L tanks containing freshwater supplied from the River Imsa. In addition, eight AquaGen and eight Imsa smolts were sampled directly from each of the two 4000-L freshwater holding tanks and acted as an untreated group. The conditions and stocking densities within these larger 4000-L freshwater holding tanks are similar to those used to hold smolts prior to being transferred to seawater cages. After each group of eight fish was held for 24 h in the smaller 100-L freshwater tanks, the water was rapidly replaced with seawater (32 parts per thousand (ppt)) and the fish were exposed for 1, 3, 8, 24, and 96 h and 2 weeks prior to sampling. Water replacement was accomplished by rapidly reducing tank volumes from 100 L to 10 L and then immediately replacing the drained freshwater with seawater. The salinity rose to that of full-strength seawater (32 ppt) within 60 min. The changes in salinity and temperature during seawater exposure paralleled the changes that hatchery-reared smolts experience during transfer from freshwater tanks to seawater cages. To determine the impact of the acute stress associated with water replacement protocol, all transfers were repeated identically using freshwater instead of seawater. This controls for the effects of low water but does not mimic the impact of the temperature change between freshwater and seawater. Both AquaGen and Imsa smolts from the freshwater replacement group developed a fungal infection just prior to the 2-week sampling point and thus fish from this time point were not used in the study.

Sampling of fish

To minimize RNA degradation, rapid tissue sampling was required, thus smolts were quickly sampled in pairs. At the appropriate time, fish were netted in pairs and exposed to a 20-L anaesthetic bath containing 100 $mg \cdot L^{-1}$ MS-222 (Sigma-Aldrich Co., St. Louis, Mo.) buffered with 200 $mg \cdot L^{-1}$ $NaHCO_3$. Blood was obtained by caudal puncture using a heparinized syringe and the sample placed on ice. Immediately after drawing blood, the first gill arches were removed and stored in RNA*later*™ (Ambion Inc., Austin, Texas) for future RNA extraction. Gill arches in RNA*later*™ were incubated overnight at 4°C before being stored at –20°C. The second gill arches were removed and placed into ice-cold SEI buffer (250 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3), frozen immediately in liquid nitrogen, and stored at –80°C for future analysis of Na^+,K^+-ATPase activity. After a total of eight fish per treatment were sampled, blood was centrifuged for 5 min at 5000 × *g*, and plasma was separated and frozen at –20°C for future analysis.

Sample analysis

Plasma Na^+ and Cl^- levels were determined using a Nova Biomedical Stat Profile Plus 9 blood gas analyzer (Nova Biomedical, Waltham, Ma.). Na^+,K^+-ATPase activity was determined spectrophotometrically using an NADH-linked assay modified from the methods of Gibbs and Somero (1990) and McCormick (1993). Gill filaments were homogenized on ice in SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH = 7.5) by hand using a ground glass homogenizer. Homogenates were centrifuged for 30 s (4°C) at 5000 × *g* to remove filaments and other insoluble material. The supernatent was used directly in the assay of enzyme activity. Gill samples were assayed for ATPase activity in the presence and absence of the Na^+,K^+-ATPase specific inhibitor ouabain (final concentration 1 mM). Samples were run in triplicate with and without ouabain, and the difference in the rate of NADH oxidation (λ = 340 nm) between the

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two conditions was used to calculate Na^+,K^+-ATPase activity. Optimal assay conditions to give maximal enzyme activity were determined as follows: 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 50 mM imidazole, 3 mM ATP, 2 mM phosphoenol pyruvate (PEP), 0.2 mM NADH, 4 U lactate dehydrogenase (LDH), and 5 U pyruvate kinase (PK), pH 7.5. Maximal enzyme activity was determined using a Hewlett Packard HP8452 diode array spectrophotometer, equipped with a thermostated cell changer maintained at 20°C with a Haake G circulating water bath. Na^+,K^+-ATPase activity was expressed as μmol ATP·mg protein^{-1}·h^{-1}. Protein content was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, Calif.), standardized with bovine serum albumin.

Design of gene-specific oligonucleotides

To assess the level of gene expression of gill *Na^+,K^+-ATPase* α-subunit, *CFTR I*, and *CFTR II* in Atlantic salmon, we utilized a semiquantitative reverse transcription polymerase chain reaction (RT-PCR) approach (Harting and Wiesner 1997), because northern blot analysis would be unlikely to distinguish between the two very similar Atlantic salmon CFTR isoforms. Gene-specific oligonucleotide primers were designed to amplify Atlantic salmon α-subunit of Na^+,K^+-ATPase, CFTR I and CFTR II.

The α-subunit Na^+,K^+-ATPase primers target the central portion of the coding region of the α-subunit and are based upon sequences from several cloned teleost homologues (white sucker, Accession No. X58629, Schönrock et al. 1991; European eel, Accession No. X76108, Cutler et al. 1995). The following primer set was used to amplify Atlantic salmon α-subunit Na^+,K^+-ATPase (product length 950 basepairs (bp)): α-Na^+,K^+-ATPase forward 5′-GAGATC-CTGGCCCGIGATGGCCC-3′ and α-Na^+,K^+-ATPase reverse 5′-AACCACATGTGGGCCACTGTCAT-3′.

The Atlantic salmon CFTR I and CFTR II oligonucleotides used in this study were designed from the 3′UTR of each gene, a region that contained several small areas that were variable and allowed the two CFTR isoforms to be distinguished from each other (Atlantic salmon CFTR I, Accession No. AF155237; Atlantic salmon CFTR II, Accession No. AF161070, Chen et al. 2001). The following primer sets were used to amplify Atlantic salmon CFTR I (product length 394 bp): CFTR I forward 5′-GAACCTTCTCCAATA-TGGTTGAAGAGGCAAG-3′ and CFTR I reverse 5′-GCA-CAGTTTTCCTTCCCCAACTCCTAAC-3′. For Atlantic salmon CFTR II (product length 444 bp), the following primer sets were used: CFTR II forward 5′-GAGATGTGG-TCTGTCCCTTTCTTTGGC-3′ and CFTR II reverse 5′-GGCCACCATGAAAAACTAAAGAGTACCTCAG-3′.

The above gene-specific oligonucleotides were synthesized by MOBIX, McMaster University, Hamilton, Ont., Canada. β-actin oligonucleotides, designed based on the sequences of human, mouse, and rat β-actin, were used as a control (product length 294 bp) (Ambion).

Semiquantitative RT-PCR

Total RNA was extracted from small amounts of gill tissue (100 mg or less) using an RNeasy® Mini kit (Qiagen Inc., Valencia, Calif.). Samples were quantitated by spectrophotometry and qualitatively assessed by gel electrophoresis using 1% agarose formaldehyde gel. For each treatment group, total RNA was extracted from six of the eight gill samples collected. First strand cDNA synthesis was performed using 1 μg of total RNA and oligo dT primer (0.5 mg·mL^{-1}) (Promega Corp., Madison, Wis.) at a final volume of 12.5 μL. This mixture was heated at 70°C for 5 min prior to adding first strand buffer (final concentration 50 mM Tris–HCl, 50 mM KCl, 4 mM $MgCl_2$, 10 mM DTT), 20 U M-MuLV Reverse transcriptase (MBI Fermentas, Hanover, Md.), 15 U RNasin® ribonuclease inhibitor (Promega), 0.8 mM dNTPs in a final volume of 20 μL). The mixture was incubated at 37.5°C for 1 h. The reaction was stopped by heating at 70°C for 10 min.

A sample of the resultant cDNA template (1 μL) was used for the PCR reaction (final concentration: 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 pmol of each gene-specific oligonucleotide, 0.8 pmol of the β-actin control oligonucleotides (Ambion), and 2.5 U of Taq polymerase (MBI Fermentas) in a total reaction volume of 25 μL). PCR reactions were carried out using two sets of primers within the same reaction (β-actin control oligonucleotide plus Atlantic salmon gene-specific α-subunit Na^+,K^+-ATPase, CFTR I, or CFTR II oligonucleotide primers). Thermal cycling was performed according to the following protocol: 1 min denaturation at 94°C, followed by 1 min annealing at 60°C, followed by a 1-min extension step at 72°C for 27 cycles. Before initiating experiments, the linear range of the PCR for each individual primer set alone and including β-actin primers was determined. These cycling parameters (27 cycles, 60°C annealing) were chosen to insure that all sets of primers resulted in reactions that were within the exponential amplification phase of the PCR for all templates. PCR products were separated according to size by gel electrophoresis in 1.5% agarose gels (1× TAE containing 25 μg ethidium bromide·100 mL^{-1} gel). The PCR products were visualized by ultraviolet illumination and quantified by densitometry using a FluorChem™ gel documentation system (Alpha Innotech Corp., San Leandro, Calif.). For each individual sample, the integrated density value obtained for the target gene-specific amplicon was divided by the signal obtained for the β-actin control amplicon producing a relative mRNA abundance value. The use of β-actin here allows for comparisons with previous studies that used β-actin as a control in Northern blot analysis to assess gill gene expression (see Madsen et al. 1995; Singer et al. 1998; D'Cotta et al. 2000). There was no significant change in the expression level of β-actin following seawater exposure or freshwater replacement compared with untreated smolts for either strain of salmon ($P > 0.05$)

Data analysis

Plasma Na^+ and Cl^- levels, gill Na^+,K^+-ATPase activity and gill *Na^+,K^+-ATPase* α-subunit, and *CFTR I* and *CFTR II* mRNA levels were analyzed using two-way analysis of variance (ANOVA) with strain and time following seawater exposure as factors. A separate two-way ANOVA examined the effects of freshwater replacement. Where necessary, dependant variable data were log-transformed to meet assumptions of constant variance within treatment group. Detailed analysis of the treatment means using Tukey's procedure was carried out only if the ANOVA revealed significant differences

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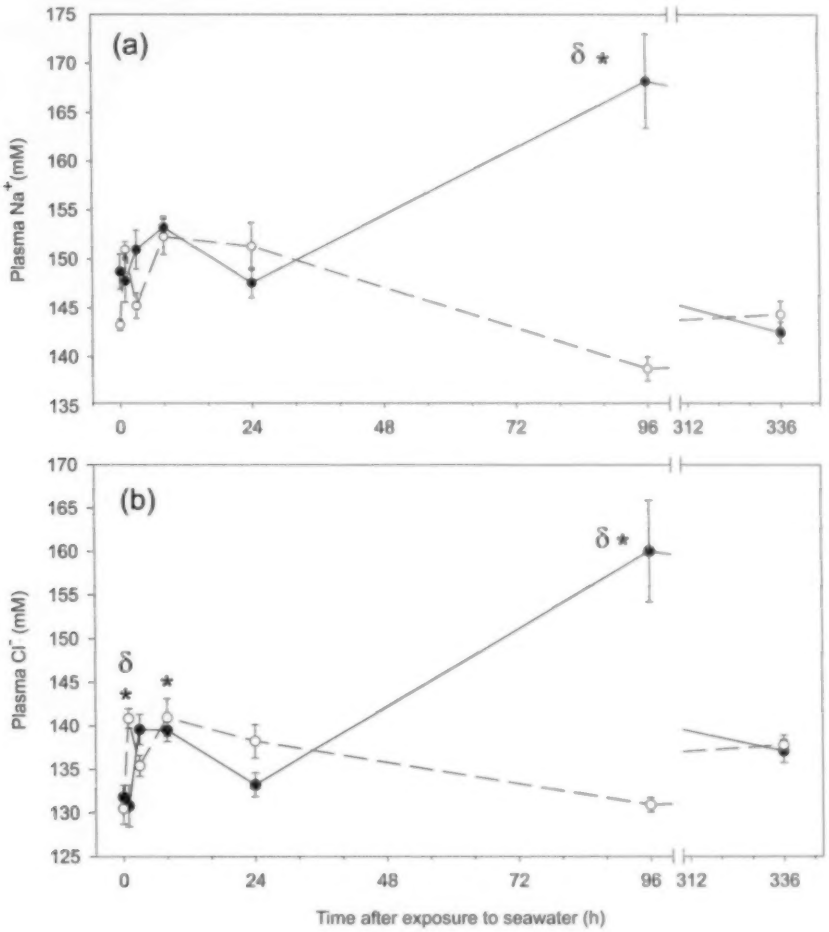
Fig. 1. Changes in plasma (*a*) Na^+ and (*b*) Cl^- levels of Atlantic salmon smolts (AquaGen (●); Imsa (○)) following seawater exposure. Values are the mean ± standard error of 6–8 fish in each group at each time interval following seawater exposure. An asterisk denotes a significant difference between a group at a specific time and the untreated group (hour = 0); the symbol (δ) denotes a significant difference between AquaGen and Imsa smolts at a specific time ($P < 0.05$, Tukey's Studentized range test, preceded by two-way ANOVA).



among main effects or interactions ($P < 0.05$). Statistical analysis was completed using SAS general linear model computer software (SAS Institute Inc., Cary, N.C.). Data are presented as mean ± standard error of the mean calculated within each treatment group. Length and weight were not significant covariants.

Results

Seawater exposure: plasma ions

Following seawater exposure, plasma Na^+ levels were significantly different between AquaGen and Imsa smolts ($P < 0.0001$). AquaGen smolts had significantly higher plasma Na^+ levels compared with Imsa smolts at 96 h following seawater exposure ($P < 0.0001$) (Fig. 1*a*). Plasma Na^+ levels in AquaGen smolts increased significantly at 96 h (168.2 ± 4.8 mM) compared with the untreated smolts (148.7 ± 1.8 mM) ($P < 0.0001$), whereas plasma Na^+ levels in Imsa smolts were never significantly different from the untreated group (Fig. 1*a*).

Plasma Cl^- levels were significantly different between AquaGen and Imsa smolts following seawater exposure ($P = 0.02$). Plasma Cl^- levels were significantly higher in the Imsa versus the AquaGen smolts at 1 h ($P = 0.02$), whereas AquaGen smolts had significantly higher plasma Cl^- levels at 96 h compared with the Imsa smolts ($P < 0.0001$) (Fig. 1*b*). Plasma Cl^- levels were significantly increased in AquaGen smolts 96 h following seawater exposure (160.0 ± 5.8 mM) compared with the untreated group (131.8 ± 1.4 mM) ($P < 0.0001$) (Fig. 1*b*). In contrast, Imsa smolts had significantly raised plasma Cl^- levels earlier at 1 h ($P < 0.04$) and 8 h ($P < 0.03$) following seawater exposure compared with the untreated group.

Seawater exposure: gill Na^+,K^+-ATPase activity

Overall, there were no significant differences in gill

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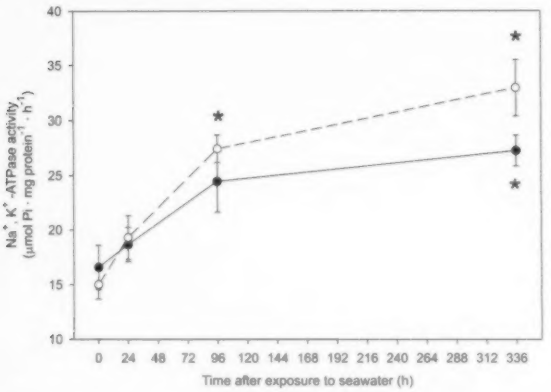
Fig. 2. Changes in Na^+,K^+-ATPase specific activity in the gill of Atlantic salmon smolts (AquaGen (●); Imsa (○)) following seawater exposure. Values are the mean ± standard error of 6–8 fish in each group at each time interval following seawater exposure. An asterisk denotes a significant difference between a group at a specific time and the untreated group (hour = 0) ($P < 0.05$, Tukey's Studentized range test, preceded by two-way ANOVA).



Na^+,K^+-ATPase activity between AquaGen and Imsa smolts at any time following seawater exposure ($P > 0.05$) (Fig. 2). Both AquaGen and Imsa smolts had significantly increased gill Na^+,K^+-ATPase enzyme activities after seawater exposure compared with the untreated group ($P < 0.05$) (Fig. 2). Activity levels rose throughout the entire time course and 2 weeks after exposure were 1.6-fold greater than in the untreated group in the AquaGen smolts and 2.2-fold greater than in the untreated group in the Imsa smolts (Fig. 2).

Seawater exposure: gill mRNA abundance

Na^+,K^+-ATPase α-subunit mRNA levels in the gills of AquaGen and Imsa smolts were not significantly different over the entire time course ($P > 0.05$). AquaGen smolts had *Na^+,K^+-ATPase* α-subunit expression levels that were significantly higher than in the untreated group through all time points following seawater exposure beginning at 24 h, increasing to 3.6-fold higher at 2 weeks ($P < 0.05$) (Fig. 3*a*). Similarly, Imsa smolts showed a significant 2.4-fold increase 2 weeks after seawater exposure compared with the untreated group, with a significant increase first apparent after 96 h ($P < 0.05$) (Fig. 3*a*).

There were near significant differences in gill *CFTR I* mRNA levels between the AquaGen and Imsa smolts (ANOVA, $P = 0.07$), but there were no significant differences when the strains were compared at any one time point ($P > 0.05$) (Fig. 3*b*). AquaGen smolts exhibited significantly increased *CFTR I* expression levels beginning at 24 h following seawater exposure rising to 2.6-fold above the untreated group after 2 weeks ($P < 0.05$) (Fig. 3*b*). Imsa salmon smolts showed a similar significant increase in *CFTR I* expression levels beginning at 96 h, increasing to 3.2-fold above the untreated group at 2 weeks ($P < 0.05$) (Fig. 3*b*).

Although gill *CFTR II* expression levels were not significantly different between AquaGen and Imsa smolts ($P = 0.12$), two-way ANOVA revealed a significant interaction between strain and time ($P = 0.001$). Further examination of *CFTR II* expression levels at specific time points showed that levels were significantly lower in the Imsa smolts versus AquaGen smolts at 2 weeks following seawater exposure ($P = 0.007$) (Fig. 3*c*). Unlike *CFTR I* expression levels, which remained elevated throughout the entire time course following seawater exposure, *CFTR II* expression was transient and was significantly elevated at 24 h by 1.3-fold for AquaGen ($P = 0.05$) and 1.4-fold for Imsa smolts ($P = 0.004$) compared with untreated smolts (Fig. 3*c*). *CFTR II* expression levels in Imsa smolts declined to levels significantly below the untreated smolts by 96 h ($P = 0.03$) following seawater exposure and were maintained at this level at 2 weeks ($P = 0.001$) (Fig. 3*c*).

Freshwater replacement

There were no significant differences in any plasma ion levels between AquaGen and Imsa smolts following freshwater replacement ($P > 0.05$)(Table 2). Both AquaGen and Imsa smolts experienced significant declines in plasma Na^+ levels ($P < 0.05$) following freshwater replacement, whereas only AquaGen smolts showed a significant decline in plasma Cl^- levels ($P < 0.05$) compared with the untreated group.

There were no significant differences in gill Na^+,K^+-ATPase activity between AquaGen and Imsa smolts at any time following freshwater replacement ($P > 0.05$) (Table 3). Neither AquaGen nor Imsa smolts had significantly increased gill Na^+,K^+-ATPase enzyme activities after freshwater replacement compared with the untreated group ($P > 0.05$) (Table 3).

There were no significant differences in *Na^+,K^+-ATPase* α-subunit mRNA levels following freshwater replacement between AquaGen and Imsa smolts ($P > 0.05$) (Table 4). AquaGen smolts showed a significant increase in *Na^+,K^+-ATPase* α-subunit expression following freshwater replacement compared with the untreated group, occurring at 24 h following freshwater replacement ($P < 0.05$), whereas the Imsa smolts showed no significant differences at any time compared with the untreated group.

There were also no significant differences in gill *CFTR I* expression between AquaGen and Imsa strains following freshwater replacement ($P > 0.05$) (Table 4). Although AquaGen smolts showed no significant increases in *CFTR I* expression following freshwater replacement, Imsa smolts had significantly higher *CFTR I* expression levels at both 24 h and 96 h compared with the untreated group ($P < 0.05$) (Table 4).

In contrast to the other two genes examined, there was a significant difference in gill *CFTR II* expression between AquaGen and Imsa smolts following freshwater replacement ($P < 0.05$) (Table 4). Overall, the AquaGen and Imsa smolts differed in *CFTR II* mRNA levels at 96 h following freshwater replacement ($P < 0.05$) (Table 4). The mRNA levels of *CFTR II* did not change in AquaGen smolts following freshwater replacement; however, Imsa smolts had a significant decrease in *CFTR II* mRNA levels at 96 h following freshwater replacement ($P < 0.05$) (Table 4).

Discussion

Seawater exposure: plasma ions

Following seawater exposure of AquaGen and Imsa smolts,

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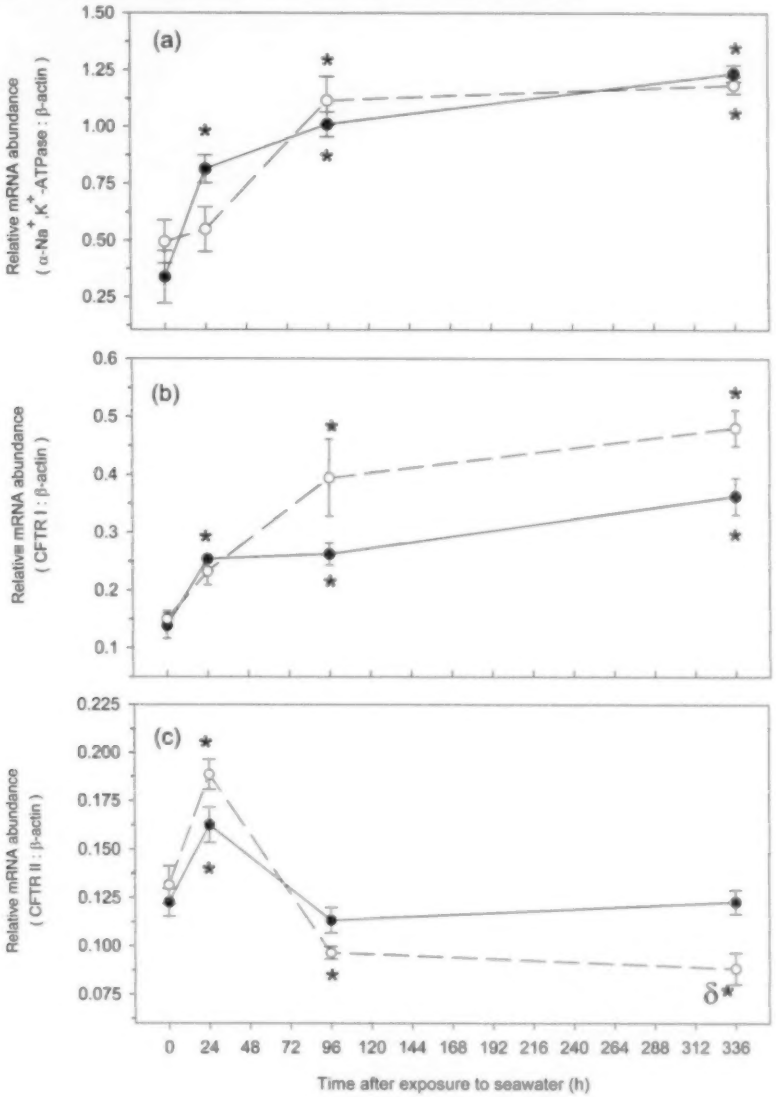
Fig. 3. Changes in relative gill (*a*) *Na⁺,K⁺-ATPase* α-subunit, (*b*) *CFTR I*, and (*c*) *CFTR II* versus β-actin mRNA abundance of Atlantic salmon smolts (AquaGen (●); Imsa (○)) following seawater exposure. Values are the mean ± standard error of 4–6 fish in each group at each time interval following seawater exposure. An asterisk denotes a significant difference between a group at a specific time and the untreated group (hour = 0); the symbol (δ) denotes a significant difference between AquaGen and Imsa smolts at a specific time ($P < 0.05$, Tukey's Studentized range test, preceded by two-way ANOVA).



plasma ion levels were significantly disrupted. Such disruptions are normal during the movement of smolts from freshwater to seawater (Hoar 1988; McCormick et al. 1989). However, we observed significantly larger disruptions in the AquaGen smolts. Most important was the significant rise in plasma Na^+ levels from initial levels of 148.7 ± 5.0 mM to 168.2 ± 11.7 mM and plasma Cl^- levels from 131.8 ± 3.9 mM to 160.0 ± 14.3 mM in the AquaGen smolts 96 h following seawater exposure. In contrast, Imsa smolts showed disturbances in plasma ion levels typical of fish fully prepared for smoltification (e.g., see D'Cotta et al. 2000). Based on the plasma Cl^- levels observed at 96 h following seawater exposure, the AquaGen smolts would be above the acceptable tolerance levels outlined in a standard seawater challenge test (Plasma chloride level < 160 mmol), whereas the Imsa smolts would not (Ugedal et al. 1998). Thus, our findings suggest that the Imsa and AquaGen smolts may not have been equally prepared to enter seawater. Because these smolts were raised under similar hatchery conditions, a genetic component may be responsible for the observed differences between AquaGen and Imsa smolts.

Others studies have reported greater increases in plasma Na^+ and Cl^- levels following seawater exposure than those observed here (Handeland et al. 1996; D'Cotta et al. 2000).

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Table 2. Plasma ion levels (mM) of Atlantic salmon smolts (strains AquaGen and Imsa) following freshwater replacement.

		Plasma concentration	
Ion	Hours	AquaGen	Imsa
Na+	0	148.7 ± 1.8 (8)	143.3 ± 0.5 (6)
	1	*137.7 ± 2.1 (7)	137.3 ± 2.9 (6)
	3	*128.7 ± 2.9 (7)	*127.2 ± 3.8 (7)
	8	140.3 ± 2.8 (7)	138.8 ± 1.4 (6)
	24	140.1 ± 1.7 (7)	136.7 ± 2.1 (7)
	96	*136.4 ± 2.3 (7)	135.5 ± 2.3 (8)
Cl^-	0	131.8 ± 1.4 (8)	130.5 ± 1.8 (6)
	1	122.0 ± 1.8 (7)	126.3 ± 2.9 (6)
	3	*113.6 ± 3.0 (7)	122.7 ± 3.5 (7)
	8	124.6 ± 2.6 (7)	126.3 ± 1.5 (6)
	24	126.1 ± 1.6 (7)	126.3 ± 1.2 (7)
	96	123.7 ± 2.0 (7)	123.7 ± 3.0 (8)

Note: Values are the mean ± standard error of 6–8 fish (number in parentheses) in each group at each time interval following freshwater replacement. An asterisk denotes a significant difference between the group from a specific time and the untreated group (hour = 0) ($P < 0.05$, Tukey's Studentized range test, preceded by two-way ANOVA).

Table 3. Na^+,K^+-ATPase specific activity (μmol Pi mg protein^{-1}h^{-1}) in the gill of Atlantic salmon smolts (strains AquaGen and Imsa) following freshwater replacement.

	Specific activity	
Hours	AquaGen	Imsa
0	16.58 ± 2.02 (8)	15.00 ± 1.30 (8)
24	12.36 ± 0.48 (8)	17.64 ± 1.72 (8)
96	16.73 ± 2.01 (8)	19.80 ± 0.94 (8)

Note: Values presented are the mean ± standard error of eight fish (number in parentheses) in each group at each time interval following freshwater replacement.

D'Cotta et al. (2000) observed that Na^+ levels reached an initial peak of approximately 185 mM after 3 h and a second later peak of approximately 170 mM at 96 h from initial Na^+ levels of approximately 140 mM. This second peak is very similar to the rise in Na^+ levels that we observed in the AquaGen smolts at 96 h following seawater exposure. In another study, Handeland et al. (1996) showed that Atlantic salmon smolts exposed to seawater had plasma Cl^- levels that increased from initial levels of 110 mM to a peak of 203 mM within 12 h, with levels remaining significantly elevated near 150 mM even after 6 d. It is possible that these differences between studies are related to the timing of the transfers, the different transfer protocols, or the differences between the strains examined.

Seawater exposure: gill Na^+,K^+-ATPase activity

Many studies have documented an increase in gill Na^+,K^+-ATPase activity in euryhaline fish upon entering seawater (for review see McCormick 1995). Atlantic salmon show a several-fold increase in Na^+,K^+-ATPase activities during parr–smolt transformation and then additional increases in gill Na^+,K^+-ATPase activities during transfer from freshwater to seawater (D'Cotta et al. 2000). Thus, Na^+,K^+-ATPase is considered by many to be the most important protein involved in gill salt secretion in teleosts. In the present study, untreated AquaGen and Imsa smolts show similar basal Na^+,K^+-ATPase activity, suggesting that both groups appear equally ready to enter seawater. In addition, both AquaGen and Imsa smolts responded similarly to seawater exposure with gill Na^+,K^+-ATPase activity rising significantly over the 2-week time course despite the fact that ion regulation differs between the strains. Similar to the pattern observed here, Shrimpton et al. (1994*a*) found that gill Na^+,K^+-ATPase activity in both wild and hatchery-reared juvenile coho salmon did not reach maximum levels until 2 weeks following seawater exposure. Likewise Seidelin et al. (2000) observed significantly increased activity from day 5 onward in seawater-transferred brown trout. However, in other studies of Atlantic salmon smolts, Na^+,K^+-ATPase levels significantly increased 4 days after seawater exposure, peaking at 11 days and subsequently subsiding (D'Cotta et al. 2000). The changes in gill Na^+,K^+-ATPase activity can only account for the well-regulated plasma ion levels in Imsa smolts and cannot explain the disruptions in plasma ions observed in AquaGen smolts.

Seawater exposure: gill mRNA abundance

Both AquaGen and Imsa smolts exhibited a several-fold increase in gill *Na^+,K^+-ATPase* α-subunit mRNA levels following seawater exposure. Gill *Na^+,K^+-ATPase* α-subunit mRNA levels have been shown to increase over the course of smoltification and following seawater exposure in both Atlantic salmon (D'Cotta et al. 2000) and brown trout (Madsen et al. 1995; Nielsen et al. 1999; Seidelin et al. 2000). However, the degree and timing of these changes differ among studies. D'Cotta et al. (2000) saw a 1.8-fold increase in gill *Na^+,K^+-ATPase* mRNA levels in Atlantic salmon at 24 h following seawater exposure, which subsequently declined slightly. Similarly, both Madsen et al. (1995) and Seidelin et al. (2000) observed transient increases in gill mRNA levels following seawater exposure in brown trout. In contrast, we observed a 2.4-fold increase in gill mRNA levels at 24 h that rose to a 3.6-fold increase relative to the untreated group by 2 weeks in AquaGen smolts. However, this increase was slightly delayed in Imsa smolts, with levels significantly increasing relative to the untreated group only after 96 h. The relationship between changes in mRNA abundance and enzyme activity is a reflection of the level of regulation. If enzyme activity is being regulated principally at the mRNA level, then changes in mRNA abundance and enzyme activity should have a 1:1 relationship. In the present study, this 1:1 relationship is observed in the Imsa smolts. In contrast, the AquaGen smolts exhibit a nearly 2:1 relationship between changes in mRNA abundance and enzyme activity. This suggests that large changes in mRNA amount are required to cause modest changes in enzyme activity in AquaGen smolts. In addition, mRNA levels in AquaGen smolts had increased significantly by 24 h, prior to the increase in enzyme activity, which is only apparent at 2 weeks. This is similar to the lag in gill activity seen in brown trout following seawater exposure (Seidelin et al. 2000). In contrast, Imsa smolts exhibited no delay between the increases in enzyme activity and mRNA levels. Taken together, these data suggest that there are substantial differ-

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Table 4. Relative gill *Na^+,K^+-ATPase* α-subunit, *CFTR I*, and *CFTR II* versus β-actin mRNA abundance of Atlantic salmon smolts (strains AquaGen and Imsa) following freshwater replacement.

		Relative mRNA abundance	
	Hours	AquaGen	Imsa
Na^+,K^+-ATPase α-subunit	0	0.358 ± 0.117 (6)	0.494 ± 0.095 (6)
	24	*0.759 ± 0.070 (6)	0.781 ± 0.060 (6)
	96	0.650 ± 0.118 (6)	0.551 ± 0.100 (6)
CFTR I	0	0.138 ± 0.022 (6)	0.149 ± 0.014 (6)
	24	0.212 ± 0.025 (4)	*0.265 ± 0.018 (6)
	96	0.196 ± 0.012 (6)	*0.286 ± 0.055 (5)
CFTR II	0	0.123 ± 0.007 (6)	0.132 ± 0.010 (6)
	24	0.140 ± 0.007 (6)	0.126 ± 0.007 (6)
	96	0.118 ± 0.007 (5)δ	*0.084 ± 0.007 (5)

Note: Values presented are the mean ± standard error of 4–6 fish (number in parentheses) in each group at each time interval following freshwater replacement. An asterisk denotes a significant difference between the indicated group and the untreated group (hour = 0), and the symbol (δ) denotes significant difference between AquaGen and Imsa smolts for a specific time ($P < 0.05$, Tukey's Studentized range test, preceded by two-way ANOVA). CFTR I and CFTR II analysis performed on log-transformed data.

ences in the mechanisms regulating gill Na^+,K^+-ATPase between these two strains.

The expression of the Cl^- channel isoforms *CFTR I* and *CFTR II* is regulated in the gills of Atlantic salmon smolts following seawater exposure, suggesting that both genes play a role during seawater adaptation. Interestingly, each Atlantic salmon CFTR isoform has a unique expression pattern and therefore may be regulated differently. The pattern of *CFTR I* expression was similar to that of *Na^+,K^+-ATPase* α-subunit with increased mRNA levels that were sustained throughout the 2 weeks following seawater exposure. In contrast, increased *CFTR II* expression was transitory with significantly elevated mRNA levels only at 24 h following seawater exposure. The physiological significance of the distinct expression patterns is unknown.

For all time points examined, *CFTR I* had a higher relative mRNA abundance than *CFTR II*. The Atlantic salmon is the first example of an organism that contains two CFTR isoforms. Atlantic salmon are tetraploid teleosts, and it is often observed that they possess multiple isoforms of the same gene (Allendorf and Thorgaard 1984). It appears that *CFTR II* expression precedes *CFTR I* expression following seawater exposure and may be involved in the initial response to increased salinity. CFTR I, on the other hand, may be involved with regulating Cl^- levels during prolonged exposure to seawater. Despite the distinct mRNA expression patterns, the two isoforms show a high degree of protein identity and are predicted to be 95% identical at the amino acid level (Chen et al. 2001). The functional properties associated with each isoform remain to be determined.

Freshwater replacement

Following freshwater replacement, the plasma ion levels of both AquaGen and Imsa smolts remained unchanged from their respective untreated group through most of the time course. The small changes in plasma ion levels that did occur are likely related to the mild acute stress associated with the freshwater replacement for both AquaGen and Imsa smolts. However, because the freshwater replacement protocol did not mimic the temperature differences that were present with seawater exposure, comparisons between the two treatments must be made with caution. Freshwater replacement caused no changes in gill Na^+,K^+-ATPase activity compared with untreated fish, nor were there any differences between AquaGen and Imsa smolts at any time following freshwater replacement. Thus the mild acute stress associated with the water replacement protocol was not capable of stimulating gill Na^+,K^+-ATPase enzyme activities. We cannot rule out the possibility that the temperature changes associated with seawater transfer cause the increases in Na^+,K^+-ATPase and *CFTR* expression, but the clearly defined role for these genes in osmotic regulation makes this unlikely.

For *Na^+,K^+-ATPase* α-subunit and *CFTR I*, the increased gill mRNA levels were modest and transient, in contrast to the larger and sustained increase following seawater exposure. For *CFTR II*, there was no significant increase in mRNA levels with freshwater replacement compared with significant, although transient, increases following seawater exposure. Interestingly, there appear to be modest differences between strains for gill *CFTR II* expression 96 h following freshwater replacement and 2 weeks following seawater exposure. The physiological significance of the differences between strains in *CFTR II* expression is not known. Based on the expression patterns of all three genes, the response to freshwater replacement likely reflects a transient mild stress response. Several studies supporting this idea have shown that both *Na^+,K^+-ATPase* and *CFTR* mRNA levels in gills are regulated by stress and (or) cortisol levels (Nichols and Weisbart 1985; Marshall et al. 1999; Shrimpton and McCormick 1999).

Origin of strain differences

Despite similar rearing conditions, the AquaGen salmon showed a significantly increased level of ion disruption following seawater exposure. These differences may be due to either inferior ability of AquaGen smolts to sense cues that normally prepare fish for seawater exposure or to AquaGen smolts being less capable of tolerating seawater exposure even when fully prepared. The physiological differences between the two studied strains are likely to be largely genetic in origin, because the fish were reared under similar hatchery conditions. Moreover, they likely arose as a result of

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(*i*) historical differences between the Namsen and Imsa populations that existed prior to domestication and (or) (*ii*) the process of domestication of the AquaGen (Namsen) strain. In a comparison of six stocks of hatchery coho salmon, McGeer et al. (1991) concluded that the differences in response to salinity challenge likely had a genetic basis, reflecting historical selective differences among the stocks. The differences observed in the present study may similarly reflect historical selective differences, particularly differences in the timing of migration. The AquaGen strain derived from the River Namsen in mid-Norway normally reach peak smoltification a week or two later than the more southern Imsa River native salmon (Heggberget et al. 1993). Moreover, differences in river topography between the larger, longer River Namsen and the much more abrupt seawater entry of the River Imsa may have selected for different physiological traits to cope with abruptness of the freshwater–seawater transition.

An alternative, but not mutually exclusive, cause of the physiological differences between the strains is domestication. Several studies have demonstrated that AquaGen salmon have diverged from wild salmon in important aspects of behaviour, including aggression, dominance, and predator avoidance, as well as growth and physiology (Fleming and Einum 1997; Johnsson et al. 2001). In fact, the same strains of AquaGen and Imsa salmon used in the current study show differences in behaviour and growth that parallel those resulting from domestication (Einum and Fleming 1997; Fleming et al. 2000).

We have shown that the three genes examined play a role during seawater adaptation. However, because no significant differences in the expression of these genes were identified between the AquaGen and Imsa smolts following seawater exposure, except for *CFTR II* levels at 2 weeks, it is likely that they have not undergone alterations that effect expression and thus do not play a role in the disruption of plasma ions in the AquaGen smolts. Other as yet unidentified genes may be responsible for the different response between AquaGen and Imsa smolts. Further studies are required to identify these candidates, which may show distinct differences in expression patterns between strains leading to the physiological differences observed here.

During seawater exposure, smolts examined in this study responded to both salinity changes and the stress of water replacement. Similarly, smolts reared for aquaculture purposes transported from hatchery freshwater tanks to sea cages would be exposed to the same changes in salinity and temperature. Thus, the observed disruptions in plasma ion levels and the associated changes in gene expression likely reflect the changes that occur during typical aquaculture practices. The AquaGen smolts examined here appear to have reduced ability to respond to the changes in salinity and temperature associated with seawater exposure.

This study has focused on comparing the physiological and molecular response of two distinct strains of Atlantic salmon that have previously shown differences in reproductive success while being reared under similar conditions. The changes in the expression of the candidate genes and activity of Na^+,K^+-ATPase examined here could only account for the response to the seawater exposure but could not account for the differences in the response between strains. In contrast, differences in Na^+,K^+-ATPase activities have been observed in studies that compared the effects of rearing conditions. Nielsen et al. (1999) observed increased Na^+,K^+-ATPase activities in wild compared with hatchery-reared brown trout during smolt development. The differences in osmoregulatory capacity between hatchery-reared and wild salmon have been attributed to rearing conditions (Shrimpton et al. 1994*a*). Similarly, Shrimpton et al. (1994*b*) suggested that the lower gill Na^+,K^+-ATPase activities in hatchery-reared coho salmon smolts versus wild smolts was a direct result of rearing conditions. These differences have been attributed to environmental cues that are likely muted in a hatchery environment. In support of this conclusion, we found no differences between AquaGen and Imsa strains in gill Na^+,K^+-ATPase activities when salmon were raised under identical conditions. However, there are clear differences in the ability of Atlantic salmon to tolerate seawater exposure, even in fish raised under identical conditions.

The rearing of different strains under hatchery conditions has the potential to lead to large differences in the physiological capabilities required for normal development of smolts. Identification of both important environmental and critical genetic components will aid in our ability to match the appropriate strain of salmon with a particular environmental condition to ensure proper growth and development. New approaches, including the use of DNA microarray technologies (see Xiang and Chen 2000 for review), will be required to identify the genes that can be used to select strains that will improve salmonid culture and reduce the disturbance of native salmonid stocks.

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Comparison of the frequentist properties of Bayes and the maximum likelihood estimators in an age-structured fish stock assessment model

Anders Nielsen and Peter Lewy

Abstract: A simulation study was carried out for a separable fish stock assessment model including commercial and survey catch-at-age and effort data. All catches are considered stochastic variables subject to sampling and process variations. The results showed that the Bayes estimator of spawning biomass is a useful but slightly biased estimator for which the frequentist variance can be estimated by the posterior variance. Comparisons further show that the Bayes estimator is better than the maximum likelihood in the sense that it is less biased and, surprisingly, has a much lesser variance. The catch simulations were based on the North Sea plaice (*Pleuronectes platessa*) stock and fishery data.

Résumé : Nous avons fait la simulation d'un modèle séparable d'évaluation des stocks de poissons qui inclut des données commerciales et des données d'inventaire sur les captures aux différents âges et sur les efforts de pêche. Toutes les captures sont considérées comme des variables stochastiques sujettes aux variations d'échantillonnage et de manipulation. Les résultats indiquent que l'estimateur bayésien de la biomasse des géniteurs est utile, mais qu'il comporte une faible distorsion; on peut déterminer la variance fréquentiste de l'estimateur à l'aide de la variance a posteriori. Des comparaisons montrent que l'estimateur bayésien est un meilleur estimateur que la vraisemblance maximale, parce qu'il comporte une erreur systématique moins importante et, de façon inattendue, une variance bien moindre. Les similuations de captures sont basées sur des données de stock et de pêche de Plies (*Pleuronectes platessa*) communes de la Mer du Nord.

[Traduit par la Rédaction]

Introduction

The first age-structured fish stock assessment methods applied were deterministic (e.g., Fry 1949; Gulland 1965; Pope 1972), and thus it wasn't possible to obtain estimates of the uncertainties of the interest parameters. Early stochastic approaches to fish stock assessment (e.g., Doubleday 1976; Fournier and Archibald 1982; Lewy 1988) were all based on the frequentist paradigm and they all used the maximum likelihood principles to estimate parameters and uncertainties.

In recent years, many approaches to fish stock assessment have been based on the Bayesian paradigm (e.g., McAllister et al. 1994; Ianelli and Fournier 1998; Virtala et al. 1998). There are several reasons for that. Assessment models often include parameters for which no or only limited information is available. In these situations it can be argued that it is better to assign prior distributions to these parameters than just assuming them to be fixed at some (arbitrary) constant values. The wish to incorporate other sources of information, such as expert opinions, is another reason to consider the Bayesian approach, where prior beliefs are expressed as a probability distribution and updated with empirical evidence to obtain a posterior distribution. Furthermore, the Bayesian approach has made it possible to analyze more realistic but complex fish stock assessment models.

The increasing popularity of the Bayesian approach has a more practical reason as well. Previously, it was not possible in general to evaluate the high dimensional integral required to calculate the posterior distribution. Simulation techniques (Metropolis et al. 1953; Hastings 1970; Tierney 1994) and fast computers have solved this problem. In fact, these simulation methods have made the Bayesian approach applicable in situations where maximum likelihood estimation is numerically impossible.

It is important to note that the estimated uncertainties from a Bayesian and a frequentist analysis are conceptually different and in principle incomparable. Whereas Bayesian uncertainties consist in prior beliefs of the analyst updated with empirical evidence, the uncertainties considered by the frequentist are based on the distribution of the estimators obtained from observations sampled from the statistical model. Furthermore, the Bayesian credibility intervals express the actual credibility related to the parameters, whereas the frequentist confidence interval is the interval that includes the estimator in a given percentage of the cases when data is sampled repeatedly from the model.

When presenting a new model and estimator, it is important to investigate their properties. For assessment methods, this is done relatively seldomly (ICES 1988; Patterson and Kirkwood 1995; NOAA 1998). In this paper we present a simple, age-structured, separable assessment model that uses

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A. Nielsen and P. Lewy.[1] Danish Institute for Fisheries Research, Charlottenlund Slot, DK-2920 Charlottenlund, Denmark.

[1]Corresponding author (e-mail: pl@dfu.min.dk).

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information from two fleets, two surveys, and additional catches without effort. The model has been applied to plaice (*Pleuronectes platessa*) in the North Sea.

The object of the present paper is to evaluate and compare the properties of a Bayesian estimator and the maximum likelihood estimators. The difficulties with the conceptual difference are avoided by considering the frequentist properties of both estimators. Frequentist properties of estimators are properties extracted from the distribution of the estimators obtained from repeated simulations of observations following the statistical model considered. This is the classical frequentist way of measuring the consistency and efficiency of estimators.

Few simulation studies previously conducted compare the different methods of estimation (Restrepo et al. 2000; Cadigan and Myers 2001). The models considered in the two papers are of the virtual population analysis type where total catch in numbers are assumed to be known without error and the uncertainties considered are connected to survey indices and commercial catch per unit effort (CPUE) data. This study assumes that total catch in numbers, survey indices, and commercial CPUE data are subject to random variation and that the statistical model used corresponds to the true model from which the observations were generated. It has also been investigated whether the estimated Bayesian credibility intervals can be used to approximate the frequentist confidence intervals of the Bayesian estimators.

Materials and methods

Data source

Data for North Sea plaice in 1988–1997 (ICES 1988) have been used as a template for this simulation study. This data set includes catch-at-age and effort data for Dutch and English commercial beam trawl fleets, survey indices for Dutch beam trawl and sole net surveys, and catch-at-age data for the combined fleet without effort data (the residual fleet). Mean weight-at-age data are the same as used by the International Council for the Exploration of the Sea (ICES). Natural mortality, M, was assumed to be known and equal to 0.1.

Model

The model is a separable model including catch-at-age and effort data for commercial fleets and for surveys. All catch observations are considered stochastic variables subject to sampling and process variations. Contrary to most virtual population analysis like models, the present model does not apply total catch-at-age, but instead a residual fleet is considered to consist of all catch data without effort information. If sampling programmes from different fleets are independent, all catch-at-age observations are independent for given numbers of fish in the sea.

All catch in numbers data are assumed to follow log-normal distributions. For the residual catches (denoted C^{res}), it is assumed that

$$(1) \qquad E(\log C^{res}_{ay}) = \log\left(\frac{F^{res}_{ay}}{Z_{ay}}(1-e^{-Z_{ay}})N_{ay}\right)$$

$$(2) \qquad V(\log C^{res}_{ay}) = \sigma^2_{res}$$

where a denotes the age, y is the year, Z is the total mortality rate, F^{res} is the partial mortality rate connected to the residual catch, E is the expected value, and V is the variance.

We model $\log(C^{res})$ instead of C^{res} because catches are positive numbers, and because it is not reasonable to assume all catches to have the same variance. Assuming a common variance on the log-catches corresponds to assuming a common coefficient of variation (CV) for all catches, which seems more reasonable. Further, it is assumed that F^{res} is multiplicative

$$(3) \qquad F^{res}_{ay} = F^{res}_{a} F^{res}_{y}$$

with $F^{res}_{y=1}=1$ to avoid overparameterization. To further reduce the number of parameters we assume that

$$(4) \qquad F^{res}_{a=A-2} = F^{res}_{a=A-1} = F^{res}_{a=A}$$

where A denotes the maximum age included. For the catches from the commercial fleets with effort data it is assumed that

$$(5) \qquad E(\log C_{fay}) = \log\left(\frac{e_{fy}Q_{fa}}{Z_{ay}}(1-e^{-Z_{ay}})N_{ay}\right)$$

$$(6) \qquad V(\log C_{fay}) = \sigma^2_f$$

where e_{fy} is the effort of fleet f in year y and Q_{fa} is the catchability. Note that we have one variance parameter for each fleet. Now we can define the total mortality rate

$$(7) \qquad Z_{ay} = M + F^{res}_{ay} + \sum_f e_{fy}Q_{fa}$$

The indices from the research surveys are also assumed to follow a log-normal distribution with

$$(8) \qquad E(\log I_{say}) = \log\left(Q^{survey}_{sa} e^{-Z_{ay}\frac{D_s}{365}} N_{ay}\right)$$

$$(9) \qquad V(\log I_{say}) = \sigma^2_s$$

where s denotes the survey, I is the observed survey index, D_s denotes the day of the year when the survey was conducted, and Q^{survey} denotes the catchability of the survey. The catches from the surveys are assumed to be negligible such that the effect on the total mortality rate, Z, can be neglected.

Initial stock size $(N_{1,y})_{y=1,\ldots,Y}$ and $(N_{a,1})_{a=2,\ldots,A}$ are selected as parameters, whereas the remaining stock sizes are considered to be deterministic functions recursively derived by the stock equation and previously defined parameters:

$$(10) \qquad N_{ay} = N_{a-1,y-1} e^{-Z_{a-1,y-1}}, a \geq 2, y \geq 2$$

This model is well suited to obtain and compare the Bayesian and maximum likelihood estimators because it is a fairly realistic model that has been applied to real data and because both estimates can be computed.

Estimation methods

For North Sea plaice fished by one commercial fleet without effort data and two fleets having effort data and two surveys, the model described contains 70 parameters

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$$\theta = (N_{a=1,y=1},\ldots,N_{a=12,y=1},\ N_{a=1,y=2},\ldots,\ N_{a=1,y=10},\sigma^2_{\text{res}},F^{\text{res}}_{a=1},\ldots,F^{\text{res}}_{a=10},F^{\text{res}}_{y=2},\ldots,\ F^{\text{res}}_{y=10},Q_{f=1,a=2},\ldots,Q_{f=1,a=9},\sigma^2_{f=1},\ Q_{f=2,a=4},\ldots,Q_{f=2,a=10},\sigma^2_{f=2},Q_{s=1,a=1},\ldots,\ Q_{s=1,a=7},\sigma^2_{s=1},Q_{s=2,a=1},\ldots,Q_{s=2,a=3},\sigma^2_{s=2}) \tag{11}$$

and 361 observations. Two estimation methods have been applied: maximum likelihood ($\hat{\theta}_{\text{ML}}$) and Bayes ($\hat{\theta}_{\text{B}}$) methods. The maximum likelihood estimates are found by maximizing the likelihood function, which is equivalent to minimizing the negative log-likelihood ℓ

$$\hat{\theta}_{\text{ML}} = \underset{\theta\in\Theta}{\operatorname{argmin}}\,\ell(\theta, C^{\text{res}}, C_1, C_2, I_1, I_2) = \underset{\theta\in\Theta}{\operatorname{argmin}} \sum_f \left(\frac{n_f \log(2\pi\sigma_f^2)}{2} + \frac{\text{SSD}_f}{2\sigma_f^2} \right) \tag{12}$$

where n is the number of observations, SSD is the sum of squared deviations from each of the five fleets, and Θ is the parameter space. The variances of these estimates are estimated by the inverse of the empirical Fisher information.

For the Bayesian method, the posterior distribution is simulated using a random walk Metropolis–Hastings algorithm. This Markov chain is denoted

$$\theta^{\text{MC}} = (\theta^{\text{MC}_j})_{j=1,\ldots,S} = (\theta_1^{\text{MC}_j},\ldots,\theta_{70}^{\text{MC}_j})_{j=1,\ldots,S} \tag{13}$$

where j denotes the step number and S is the number of steps for which the chain is simulated. This chain is constructed to have the posterior distribution as equilibrium distribution. The model parameters are estimated by the averages

$$\hat{\theta}_{\text{B}} = \frac{1}{S}\sum_{j=1}^{S} \theta^{\text{MC}_j} = \left(\overline{\theta_1^{\text{MC}}},\ldots,\overline{\theta_{70}^{\text{MC}}}\right) \tag{14}$$

where $\hat{\theta}_{\text{B}}$ denotes the Bayesian estimate. The posterior variances of parameters are estimated by the empirical variances in the chain.

For the Bayesian analysis, uniform priors are assigned to all parameters to obtain the estimates most comparable to maximum likelihood estimates. The ranges of these uniform distributions were in all cases chosen wide enough to include all possible values of the parameters. The marginal stock size parameters were each assigned a uniform distribution between 0 and an unrealistic high stock size of 1×10^{10}. The mortality parameters F^{res}_a, F^{res}_y, and Q_{fa} were assigned a uniform distribution between 0 and 10. The variance parameters of the log-transformed observations are assigned a uniform prior between 0 and 10. A value of 10 corresponds to a CV of about 148 for the original observations, which covers the possible range.

The simulation study

To study the properties of an estimator, the true parameter values have to be known. We want to apply parameters for a plaice-like stock. This was obtained by calculation of the maximum likelihood estimates using the model and the North Sea plaice data. These estimates were chosen as the "true parameter values". Based on these parameter values, new sets of observations were generated from the probability distribution of catches with exactly the same dimensions as the real plaice data set (and the same missing values). For these sets of observations, we know the true parameter values, and we know that these sets of observations follow the model. For each of these sets of observations, all model parameters have been estimated using Bayes (the posterior mean) and maximum likelihood estimators, ML. These estimates are respectively denoted $\hat{\theta}_{\text{B}}^{(d)}$ and $\hat{\theta}_{\text{ML}}^{(d)}$, $d = 1,\ldots,D$, where d denotes the data set index and D is the number of data sets generated. Further, for the Bayes method, the posterior standard deviation, $s_{\theta_{\text{B}}}^{(d)}$, has been calculated, whereas the standard deviations of the estimators, $s_{\hat{\theta}_{\text{ML}}}^{(d)}$, have been calculated for the maximum likelihood method. The structure of the simulation study is summarized in Fig. 1.

The bias of the two methods is estimated by

$$\widehat{\text{bias}}_{\hat{\theta}} = \frac{1}{D}\sum_{d=1}^{D}(\hat{\theta}^{(d)} - \theta_0) \tag{15}$$

To visually compare the bias estimates (which are on very different scales) the relative bias, $\widehat{\text{bias}}_{\hat{\theta}}/\theta_0$, is considered. It is an advantage that an estimator is unbiased or only slightly biased, but if the estimator has a huge variance, then we still have a small probability of getting estimates close to the true value. The mean squared error (MSE), defined as $E(\hat{\theta} - \theta_0)^2 = \text{var}(\hat{\theta}) + \text{bias}_{\hat{\theta}}^2$, is a measure with the characteristic that small values imply that both bias and variance are small. MSE is estimated by

$$\widehat{\text{MSE}}_{\hat{\theta}} = \frac{1}{D}\sum_{d=1}^{D}(\hat{\theta}^{(d)} - \theta_0)^2 \tag{16}$$

Bias and MSE were calculated for all parameters, for spawning stock biomass (SSB) by year, and for average fishing mortality over ages by year. Furthermore, a status quo prediction of SSB one year ahead was carried out assuming that fishing mortality in the prediction year equals that of the last data year. SSB was calculated using the stock weight at age data for North Sea plaice. For the prediction year, the weights for the last data year was used.

We experienced some technical problems in setting up this simulation study. To sample the posterior distribution we used a random walk Metropolis–Hastings sampler. The initial value was the maximum likelihood estimate $\hat{\theta}_{\text{ML}}^{(d)}$. The proposal distribution was (apart from bounds on the parameters) a mixture between a normal distribution $N(\theta_n, I(\hat{\theta}_{\text{ML}}^{(d)})^{-1})$ and a "heavy-tailed" (Cauchy) distribution.

We tried the Metropolis–Hastings sampler, but in a few cases, the maximum likelihood estimator seemed to give really poor estimates. In these cases we experienced a very poor mixing of the Markov chain, as this sampler is very dependent on the maximum likelihood estimates and the estimated Hessian. To solve this problem we did the following. Using the data for North Sea plaice the covariance matrix, $I(\hat{\theta}_{\text{ML}}^{(0)})^{-1}$, of the maximum likelihood estimates was calculated. This covariance matrix was used in the proposal distribution for all steps in the simulations. Then, we ran our sampler for 1000 steps, again using the real data set. From this short run, we saved the last step to be used as initial value in all the samplers. The new sampler uses the same

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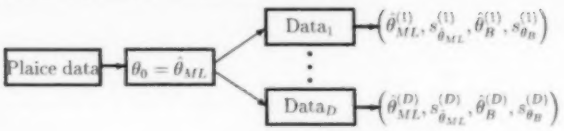
Fig. 1. Diagram of the simulation study.



initial values and the same covariance matrix for all the data sets. The initial values are close (but not equal) to the true values. The covariance matrix is known to give good mixing properties for the real data set. The samplers were greatly improved by this, but we still had to run the samplers for a very long time to be convinced that they had all reached their equilibrium distribution.

We generated 210 data sets. For each data set, we ran the Markov chains for a burn-in period of 1×10^5 steps, which were discarded. Then, we ran the chains for 1×10^6 steps, but for the estimation we only used every hundredth step for computational convenience. This means that the number of steps in the chain used was 10 000. Two of the data sets were excluded from the rest of this study because the maximum likelihood method did not converge. So, we effectively have $D = 208$. It would have been preferable if the number of sampled data sets could have been increased, but the computer time the Markov Chain Monte Carlo (MCMC) estimation required for each data set limits the number of data sets used.

Results

The two estimation methods have been applied to the real data set for plaice in the North Sea. We found that SSB estimates from the two methods are far from identical. The maximum likelihood estimates are in all years substantially above Bayes estimates (Fig. 2). We have added the ICES working group estimates from the extended survives analysis (XSA) to the plot to illustrate that Bayes estimates from this simple model are actually very similar to the XSA estimates.

We found that SSB estimates are biased for both the maximum likelihood and Bayes estimators (Fig. 3). Bayes estimates are less biased as Bayes estimates are underestimated by about 5–10%, whereas the maximum likelihood estimates are overestimated by about 15–20%. Especially, this also applies to SSB predicted in 1998. It is important to emphasize that the confidence intervals on the graph (Fig. 3) are not the percentiles of the empirical distribution of the bias, but confidence intervals for the bias estimator (average bias). This implies that the length of these intervals are inversely proportional to $\sqrt{D}$, and because of this it may appear that there is no (visible) uncertainty on Bayes estimates. Obviously, this is not the case; let's consider the following example. The true value of SSB in 1992 in this simulation study was 3.61×10^5 t and the empirical standard deviation of the bias was 1.68×10^4 t. This gives us a CV around 5%. The graph is useful to conclude if the bias is significant or not, but the confidence intervals must be interpreted carefully.

MSE is shown in Table 1. It appears from the table that the MSE of the Bayes estimator of SSB is much smaller than MSE of the maximum likelihood estimator. Actually, the MSE of the Bayes estimator is less than 5% of the MSE of the maximum likelihood estimator! Similarly, the results

Fig. 2. Spawning stock biomass (SSB) estimated from the real plaice data set by maximum likelihood (broken line), Bayes (solid line), and extended survivors analysis (○).

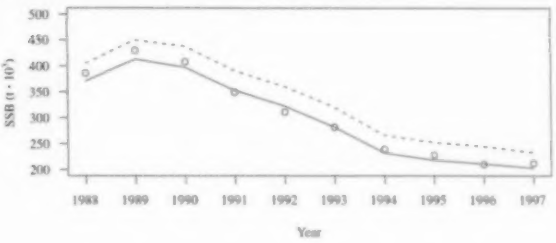
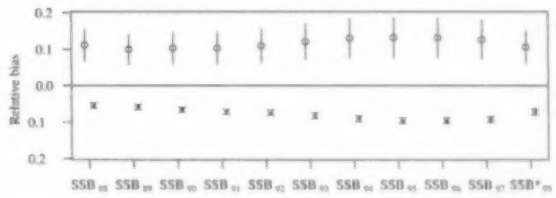


Fig. 3. Relative bias of the spawning stock biomass (SSB) estimated by the maximum likelihood (○) and Bayes estimators (×). SSB_{98} is a predicted value. The vertical lines show the approximated 95% confidence intervals of the bias estimates.



show that standard deviation of the Bayes estimator is only about one fifth of the standard deviation of the maximum likelihood estimator. The Bayes estimator is thus superior to the maximum likelihood estimator, as the former is both less biased and more accurate. Further, it is a conservative estimate in the sense that it constitutes a lower limit.

The Bayes estimator of recruitment is unbiased and more accurate (Fig. 4*a* and Table 1). The only exception is the recruits in the last year, $N_{a=1,y=97}$. This is poorly estimated by both estimators and MSE is almost the same, indicating that the estimators are equally poor for that year. In general, maximum likelihood estimates of stock size seem to be too large, whereas Bayes estimates seem to be unbiased.

We found that the results for recruitment and spawning stock biomass do not apply to all the fishing mortality parameters (Fig. 4*b*). The figure shows that for F averaged over age groups 2 to 10, $\overline{F}_{2-10}$, used by the working group (ICES 1998), the maximum likelihood estimates are less biased than Bayes estimates, which are severely biased with a relative bias of 15–20%. However, again MSE (Table 1) indicates that Bayes in general is a bit better estimator than the maximum likelihood. We found that Bayes estimates of $\overline{F}_{2-6}$ with a relative bias of 5–10% are less biased than $\overline{F}_{2-10}$, but still more biased than the maximum likelihood estimates of $\overline{F}_{2-6}$ (Fig. 4*c*). For $\overline{F}_{2-6}$, MSE of Bayes is about the half of the MSE of the maximum likelihood estimates, indicating that Bayes provides the best estimator for this average F.

A closer inspection of results shows that the reason why Bayes is a better estimator of $\overline{F}_{2-6}$ than of $\overline{F}_{2-10}$ is that for age groups 7–10 the Bayes estimator is severely biased with relative large values of MSE. For age groups less than seven Bayes is a better estimator than the maximum likelihood.

Figure 5 and Table 2 show that the Bayes estimator of the

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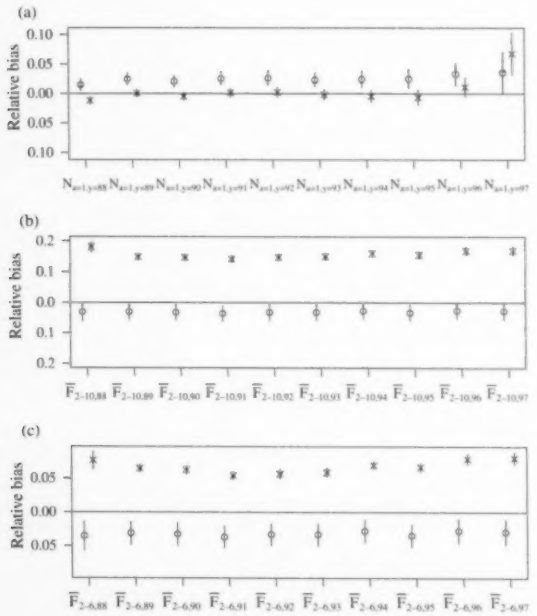
Table 1. Mean square error (MSE) of Bayes and maximum likelihood (ML) estimates of spawning stock biomass (SSB), average F, and recruitment.

	$SSB \cdot 10^{-8}$		$\bar{F}_{2-6} \cdot 10^{3}$		$\bar{F}_{2-10} \cdot 10^{3}$		$N_{a=1} \cdot 10^{-8}$	
MSE	Bayes	ML	Bayes	ML	Bayes	ML	Bayes	ML
1988	9.1	185	1.5	2.6	3.6	3.7	9.2	23
1989	11	196	0.7	1.8	2.0	2.6	4.5	11
1990	12	207	0.7	1.8	2.1	2.6	4.8	11
1991	11	167	0.7	2.3	2.6	3.5	4.4	11
1992	9.9	155	1.1	2.9	3.7	4.7	6.1	14
1993	9.7	148	1.1	3.1	4.0	5.0	3.3	6.1
1994	8.0	120	1.3	2.9	4.1	4.7	3.9	7.0
1995	7.9	112	1.3	3.1	4.0	4.8	17	26
1996	7.5	103	1.6	2.9	4.0	4.3	18	27
1997	6.7	88	1.8	3.2	4.3	4.4	38	37
1998*	5.1	56						
Average	8.9	139.7	1.2	2.7	3.4	4.0	10.9	17.3

*Predicted values.

Fig. 4. (*a*) Relative bias of the number of recruits, (*b*) $\bar{F}_{2-10}$, and (*c*) $\bar{F}_{2-6}$, estimated by maximum likelihood (○) and Bayes (×). The vertical lines show the approximated 95% confidence intervals of the bias estimates.



variances of catches related to the most important fleets (the residual fleet and Dutch beam trawlers), i.e., σ^2_{res} and $\sigma^2_{f=1}$, is much better than the maximum likelihood estimator because the Bayes estimator is less biased and has a smaller MSE. This may indicate that Bayes estimators provide better estimates of the uncertainty related to catch predictions.

Application of posterior credibility intervals as confidence intervals of the Bayes estimator of SSB

The standard deviation in the posterior distribution is conceptually different from the standard deviation of the maximum likelihood estimator. The former is the standard deviation of the (partly subjective) distribution of the analyst's knowledge of the parameters, given fixed observations. The standard deviation of the maximum likelihood estimator, however, is the standard deviation in the distribution of the maximum likelihood estimator when the observations are considered random variables. Apart from that the two quantities (and the connected probability distributions) are asymptotically identical, we do not know anything about the small sample properties. From a frequentist point of view it is interesting to investigate if the Bayesian credibility intervals can be given a frequentist interpretation as confidence intervals of the estimator. In the following, this has been investigated for SSB by comparing the posterior distribution of SSB with the empirical distribution of the Bayes estimate of SSB.

Fig. 5. Relative bias of the variance parameters estimated by the maximum likelihood (○) and Bayes estimators (×).

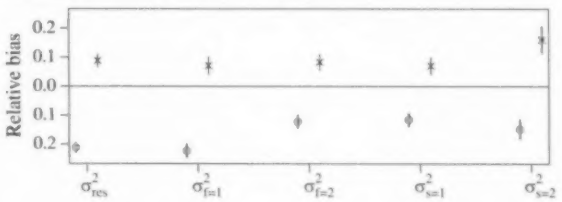


Table 2. Mean square error (MSE) of Bayes and maximum likelihood (ML) estimates of variance parameters.

		$MSE \cdot 10^{4}$	
MSE	True value	Bayes	ML
σ^2_{res}	0.077	2.1	3.6
$\sigma^2_{f=1}$	0.027	0.4	0.6
$\sigma^2_{f=2}$	0.10	4.6	4.3
$\sigma^2_{s=1}$	0.22	23	21
$\sigma^2_{s=2}$	0.27	100	63

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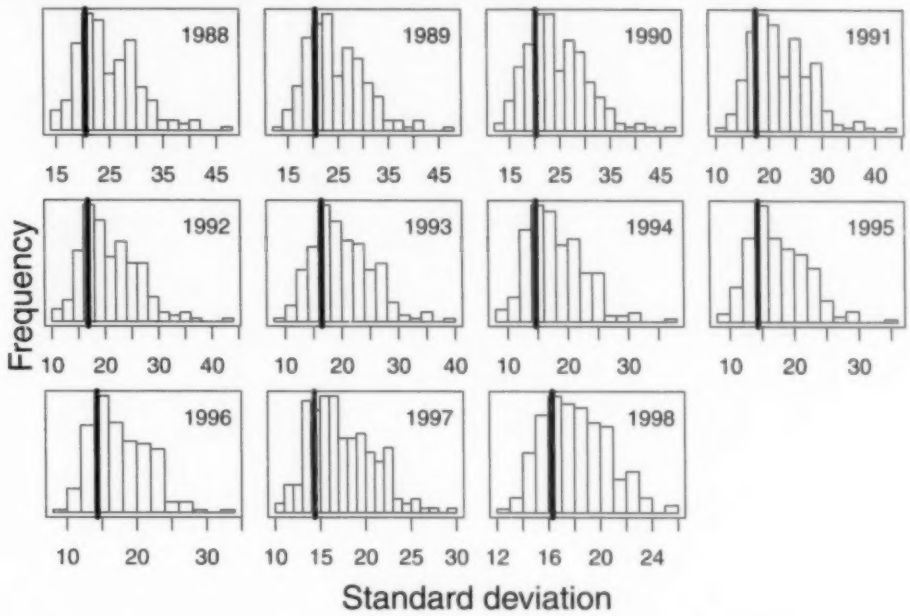
Fig. 6. Histograms of the empirical distributions of the posterior standard deviations of spawning stock biomass (SSB). The vertical bold lines indicate the empirical standard deviations of the Bayesian SSB estimates.



First, the empirical standard deviation of Bayes SSB estimator, SSB_B,

$$\sqrt{\frac{1}{D-1}\sum_d (\widehat{SSB}_B^{(d)} - \overline{\widehat{SSB}}_B)^2} \quad (17)$$

is compared with the histogram of the empirical distribution of the posterior standard deviation of SSB (Fig. 6).

It appears that the standard deviations of Bayes estimators of SSB are close to the mode of the posterior of distribution of standard deviations, indicating that the posterior standard deviation may be a useful estimate of the standard deviation of the Bayes estimator. This also indicates that the credibility intervals based on the posterior distribution of SSB may be used as confidence intervals. This is further investigated below.

The frequentist interpretation of a $(1 - \alpha)\%$ confidence interval means that when observations are sampled from the statistical model given the estimated parameters, the probability of getting an estimate outside the interval should be smaller than α. The interpretation of a Bayesian credibility interval is more straightforward. The $(1 - \alpha)\%$ credibility interval simply expresses the interval where the analyst believes the true parameter is located with a probability of $1 - \alpha$.

If the posterior distribution can be approximated by the distribution of the Bayes estimator in the frequentist sense, then the $\alpha\%$ quantile in the posterior distribution should be lower than the true value, θ_0, with probability α. This should hold for any $\alpha\%$ quantile. This can also be expressed in the following way: the distribution of the probability $P(\theta \leq \theta_0|\text{Data})$, should be the uniform(0,1) (lower than α with probability α).

We know that the Bayes estimator is biased (but much less than the maximum likelihood estimator). With a biased Bayes estimator, it is expected that the posterior distribution is biased as well. There is little doubt that the credibility intervals do not cover the right area in a frequentist sense. Instead, the bias-corrected posterior distribution is considered for investigation if the posterior distribution function of SSB, $P_{\text{bias corr.}}(SSB \leq SSB_0|\text{Data})$, is uniform. This is done using Q–Q plots (Fig. 7).

The empirical distributions of the bias-corrected posterior probabilities are not completely uniformly distributed, but the small and large quantiles of the distributions seem to be similar (Fig. 7). Focusing on the coverage of the 95% interval, the number of times when the true SSB value was not covered by the 95% bias corrected credibility interval was calculated (Table 3).

For all years, the true SSB lies outside the credibility interval in less than 5% of the cases. This indicates that the 95% credibility interval of SSB is a good but slightly wider approximation of the 95% confidence interval.

Discussion

The simulation study was conducted to analyze and compare the properties of the Bayesian posterior mean and maximum likelihood estimators of stock parameters in the model presented. Other Bayesian estimators, such as the posterior median or decision based criteria could have been used. However, we have chosen to consider the posterior mean, simply because it is by far the most commonly used Bayesian estimator. The posterior distribution considered here is proportional to the likelihood function because uniform priors were used for all parameters. Both estimators are thus based on the likelihood function: the Bayes estimator is the mean, whereas the maximum likelihood estimator is the

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Fig. 7. Q–Q plot of the empirical distribution of the bias-corrected posterior probability of $P(\mathrm{SSB} \leq \mathrm{SSB}_0|\mathrm{Data})$ versus the uniform(0,1) distribution. Each plot is based on 208 observations.

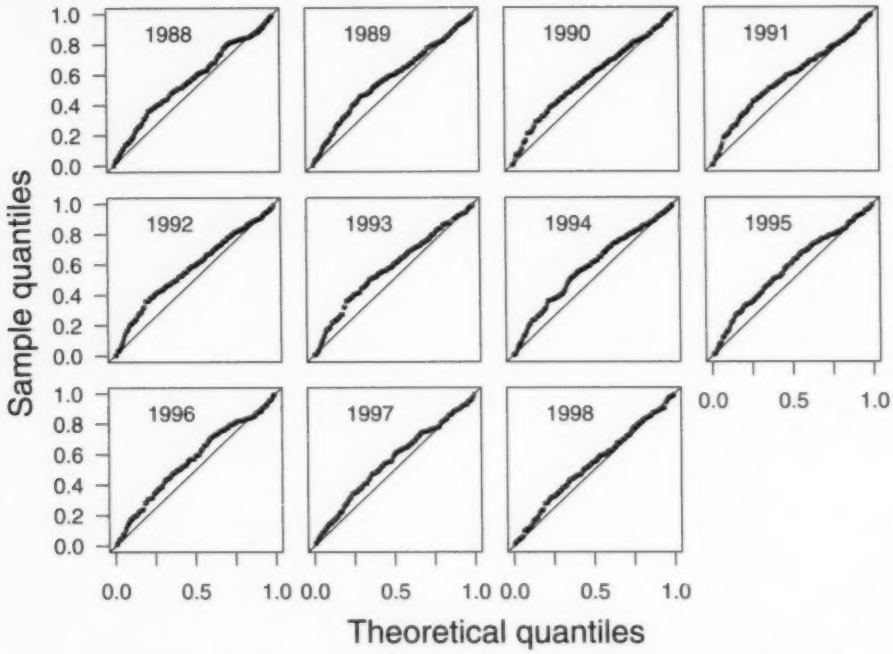

Table 3. Number of times (of 208) when the true spawning stock biomass (SSB) was not covered by the 95% bias corrected credibility interval (CI).

	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998*
No. outside 95% CI	6	6	9	10	5	10	8	8	7	5	9

*Predicted value.

mode of the normalized likelihood function. Therefore, only minor differences between the estimators are expected. Surprisingly, significant differences were found, as the Bayes estimator of spawning stock biomass, recruitment, and to some extent, of fishing mortality, was shown to possess substantially better frequentist properties than the maximum likelihood estimator. It is also indicated that Bayes estimators may provide better estimates of the uncertainty related to catch predictions.

Further, it has been shown that the posterior credibility intervals of spawning biomass can be given frequentist interpretations as confidence intervals for the Bayes estimator. This is because the posterior distribution of spawning biomass has been shown to be a reasonable approximation of the distribution of the Bayes estimator. In this case, conclusions based on the credibility intervals would thus be the same as those based on the confidence intervals.

The spawning stock biomass estimates from the real plaice data set showed the same trend as the simulated estimates. The maximum likelihood estimates were in all years substantially above the Bayes estimates. From the simulation study, we know that this difference is coerced by the estimation methods and that the unbiased estimates are probably between the two sets of estimates, but closest to the Bayes estimates.

Because most age-structured fish stock assessment models are based on the same nonlinear equations as used here, it may be reasonable to believe that similar results apply to other fish stock assessment models for other stocks. A comprehensive study of the properties of a model and the estimators also needs to include analysis of the properties of forecasts of recruitment, spawning biomass, and catches. Except for forecasting of spawning biomass one year ahead, this has not been considered here. For the forecast mentioned, it has been shown that the Bayes estimator again is superior to the maximum likelihood. In general, the properties of forecasts of catches and spawning biomass several years ahead will depend heavily on the properties of the recruitment forecasts.

It is not the authors intentions generally to encourage the application of Bayesian inference in fish stock assessment. We feel that the lack of objectivity is a key problem in a scientific context. But this does not prevent us from using Bayesian estimators if they are superior to other estimators available. However, the Bayesian approach may be useful to evaluate the effect of uncertainty on parameters otherwise assumed constant, such as natural mortality.

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Fertilization dynamics in rainbow trout (*Oncorhynchus mykiss*): effect of male age, social experience, and sperm concentration and motility on in vitro fertilization

N.R. Liley, P. Tamkee, R. Tsai, and D.J. Hoysak

Abstract: We examine aspects of the fertilization dynamics of rainbow trout (*Oncorhynchus mykiss*) that may play a role in determining reproductive success of males of different age and status competing for spawning. There were no differences in the gonadosomatic indices and relative yields of milt of adult (3-year) and precocious (1-year) male rainbow trout collected from a wild population. The concentration of sperm in the milt of precocious males was higher than that of adult males. The duration of sperm motility was similar in the two groups of males and increased over the period of sampling. Interaction of a male with a nesting female caused an increase in milt yield, but did not affect sperm concentration, sperm motility, or fertilization rates. There was a sharp decline in fertilization rate 20 s or more after activation of the sperm or eggs by fresh water. Exposure to milt suspension for as little as 0.5 s resulted in fertilization of <27% of eggs. The short gamete longevity and the speed with which fertilization occurs indicate that the timing and position of sperm release may play a critical role in determining the reproductive success of males in competition for spawning with a single female.

Résumé : On trouvera ici un examen des aspects de la dynamique de la fertilisation chez la Truite arc-en-ciel (*Oncorhynchus mykiss*) qui peuvent jouer un rôle dans la détermination du succès de la reproduction chez les mâles d'âges et de statuts différents qui sont en compétition durant la fraye. Il n'y avait pas de différence dans les indices gonadosomatiques et les productions relatives de laitance entre les truites mâles adultes (3 ans) et précoces (1 an) tirées d'une population sauvage. La concentration des spermatozoïdes dans la laitance était plus élevée chez les truites mâles précoces que chez les adultes. La durée de la motilité des spermatozoïdes était semblable dans les deux groupes et elle s'est accrue durant la période d'échantillonnage. L'interaction d'un mâle avec une femelle au nid causait une augmentation de la production de laitance, mais n'affectait ni la concentration, ni la motilité des spermatozoïdes, ni les taux de fertilisation. Il y avait un brusque déclin du taux de fertilisation 20 s ou plus après l'activation des spermatozoïdes ou des œufs par l'eau douce. L'exposition d'une suspension de laitance pendant aussi peu que 0,5 s résultait en une fertilisation de <27 % des œufs. La longévité courte des gamètes et la vitesse de la fertilisation indiquent que le moment de la libération du sperme et la position du mâle jouent sans doute un rôle critique dans la détermination du succès reproducteur de mâles qui cherchent à frayer avec une même femelle.

[Traduit par la Rédaction]

Introduction

There has been a long history of interest in sperm biology of fish. Scott and Baynes (1980), Linhart et al. (1991), and Billard (1992) review much of the available information on the morphology, biochemistry, and metabolism of the sperm of teleost fishes. The motivation for much of this work has been its relevance to the storage and handling of sperm used in in vitro fertilization procedures, particularly in the hatchery culture of salmonids and cyprinids. More recent interest has been concerned with the implications of gamete biology for sexual selection under natural spawning conditions (Gage et al. 1995; Stockley et al. 1997). The influence of behavioral competition and fertilization dynamics on male reproductive success is of particular interest in species in which males exhibit alternative reproductive tactics (Taborsky 1998). Male success may depend upon the timing and position of sperm release, ability of the sperm to compete with those of other males, and on the number of sperm released. A number of studies have identified differences in the number and quality of gametes produced by males exhibiting alternative reproductive tactics. For example, relative to body size, parr Atlantic salmon, *Salmo salar*, have larger testes and produce greater numbers of sperm and volumes of stripped ejaculate than anadromous males (Gage et al. 1995). In addition, more sperm were motile in parr ejaculates, and these sperm lived longer than anadromous male sperm.

Most information on the relationship between sperm concentration and motility and fertilization in salmonids is

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N.R. Liley,[1] P. Tamkee, R. Tsai, and D.J. Hoysak. Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada.

[1]Corresponding author (e-mail: liley@zoology.ubc.ca).

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derived from in vitro "dry" fertilization studies (Scott and Baynes 1980), in which eggs are mixed with undiluted milt or milt diluted with a variety of "salines" (Billard 1983) prior to the addition of fresh water. These studies provide no information as to the impact of sperm density in a natural spawning event. For in vitro trials in which gametes of sockeye salmon, *Oncorhynchus nerka*, were mixed simultaneously with the addition of a relatively large volume of water (conditions approximating the natural spawning situation) there was a marked decline in fertilization rate with decreasing concentration of sperm (Hoysak and Liley 2001).

The investigation described here is part of an examination of mating competition among male rainbow trout, *Oncorhynchus mykiss*, from a population spawning in the inlet to Pennask Lake, British Columbia (B.C.). In Pennask River the majority of the male and female spawning rainbow trout are 3-year-old "adults" (3 year). A large number of smaller, sexually mature, 1- and 2-year-old "precocious" males are also present in the spawning area. Observations in the laboratory, and of a natural spawning population in the outlet to Garibaldi Lake, B.C., reveal that although several adult and precocious males may attend a nesting female, one adult male becomes dominant and attempts to drive off other males. After several hours of nesting and courtship activity, the pair simultaneously release eggs and milt into the nest. One or more subordinate males may rush in and release milt alongside the spawning pair.

The outcome of competition among males may be influenced by the "priming response", an increase in plasma concentrations of gonadal steroids and gonadotropin, and an increase in spermiation (the generation of milt within the gonads) as a result of interaction with a nesting female (Olsén and Liley 1993). There is evidence from laboratory and field studies that the priming response depends upon social status, and is most pronounced in dominant males (Liley et al. 1993; Liley and Kroon 1995).

In this study we examine a number of aspects of rainbow trout fertilization dynamics potentially relevant to our attempts to identify the determinants of reproductive success of males of different age, size, and status competing for spawning. We first describe the characteristics of milt production and sperm motility in adult and precocious male rainbow trout from a natural population. Second, we compare the motility of sperm and the proportion of eggs fertilized by different concentrations of sperm, from "primed" and "unprimed" adult males. Third, because any differences in the time of release of gametes by females and males may be critical in determining the outcome of a competitive spawning, we examined (*i*) the duration of viability of sperm and eggs following activation by fresh water; and (*ii*) the speed with which fertilization occurs.

Materials and methods

Rainbow trout were obtained from a population spawning in the inlet to Pennask Lake 50 km east of Merritt, B.C. (Liley et al. 1986). Spawning commences in early June, peaks in the third or fourth week of June, and persists into early July. Fish were collected 13 June 1997 (experiment 1), 8 June 1998 (experiments 2–4), and 16 June 1999 (experiment 5). In all except experiment 5, fish were transported immediately to the laboratory in Vancouver, where males and females were separated and placed in holding tanks (800–1600 L) supplied with a constant flow of dechlorinated city tap water at 9–10°C. All fertilization and motility studies in Vancouver were conducted in the water supplying the holding tanks. Only fish without injuries, fungus, and fin wear were used to ensure that all fish were of comparable physical condition and had not already participated in spawning. At collection all males were spermiating (milt could be expressed by gentle pressure on the abdomen); females were unovulated: the abdomen was firm and eggs could not be obtained under pressure. In the laboratory, females were checked for ovulation every two days. Within two days of arrival in the laboratory, fish were weighed and labelled with a small numbered plastic tag attached by monofilament loop through the dorsal musculature immediately behind the dorsal fin. Fish were anaesthetized in 0.05% 2-phenoxyethanol prior to handling procedures.

The density of sperm in milt varies considerably among males; consequently, it was necessary to control the concentration of sperm in in vitro fertilization trials. Bouk and Jacobson (1976) demonstrated a strong correlation between spermatocrit (the proportion of milt volume occupied by packed sperm cells) and the concentration of sperm cells determined by hemacytometer. (Bouk and Jacobson (1976) recorded a mean concentration of 18.733×10^6 spermμL^{-1} milt in steelhead trout, the anadromous form of rainbow trout.) Thus, spermatocrit provides a reliable, rapid estimate of sperm concentration in milt. In experiments 2 to 5, spermatocrit was measured for each milt sample by filling two capillary tubes with milt and centrifuging them for 10 min in a hematocrit centrifuge. The average of these two values gives the proportion of milt occupied by sperm cells. Aliquots of milt calculated to contain 20 µL of packed sperm were used in all in vitro fertilization trials. In experiment 1 spermatocrit was measured only once.

Following a fertilization trial, eggs were transferred to an incubator tray (9 cm diameter) and placed in slowly flowing water. Dead eggs were removed and recorded at 2- to 3-day intervals. After 3–4 weeks, developing eggs were clearly eyed and were counted.

Experiment 1: gonadosomatic index, milt yield, spermatocrit, and sperm motility of adult and precocious males

In 1997, samples of milt for determination of spermatocrit and sperm motility on day 0 (3 or 4 days after collection), day 8, and day 16 of the sampling period were collected from 10 adult males and 12 precocious males. Samples were drawn directly from the genital papilla into a microhematocrit tube. Care was taken to avoid contamination with urine. A drop of milt was tested for motility (see below) within 5 min of collection, and the remaining sample was centrifuged to determine spermatocrit.

Motility was assessed by a procedure similar to that of De Fraipont and Sorensen (1993). A drop of milt (<1 µL) was placed in a 0.5-mL microcentrifuge tube. The sperm were activated by injecting 0.4 mL of water into the tube. Simultaneously, a timer set to give a signal at 10 s was started. Within 3–4 s, a 50-µL sample was drawn into a pipette and released under a cover slip placed halfway over the depression

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in a 1-mm welled glass slide mounted on a compound Leitz Wetzlar light microscope (100× magnification). This procedure was repeated twice for each sample. Two measures of motility were taken. (*i*) Proportion of sperm motile 10 s after activation: only forwardly moving sperm were judged to be motile; vibrating sperm were assessed to be nonmotile. The proportion of sperm motile was ranked on a 0–5 scale: 5 = >95% motile; 4 = 75–94% motile; 3 = 30–74% motile; 2 = 10–29% motile; 1 = <10% motile; 0 = no activity. (*ii*) The duration of sperm motility: the time from activation until <5% of sperm remain motile. (Note: motion of the fluids on the microscope stage make it difficult to make a reliable assessment of sperm motility less than 10 s after activation.)

Milt yield (weight of strippable milt as a percentage of body weight) was obtained from separate groups of adult (n = 10) and precocious males (n = 12) in 1997. Males were "stripped" by abdominal massage. Milt was expelled directly onto filter paper in a previously weighed plastic petri dish. The sample was covered, and weighed within 2 h of collection.

Gonadosomatic index (GSI: weight of testes as a percentage of total body weight) was determined from 12 precocious and 19 adult males obtained in 1998. Males were allowed to die in the anaesthetic and weighed, and the testes were removed and weighed.

Experiment 2: the effect of priming and sperm concentration on fertilization rate

In this experiment, we determined in vitro fertilization success in trials in which eggs from a female were mixed separately with different concentrations of sperm from a pair of adult males, one male primed by exposure to an actively nesting female for up to 3 h, the other member of the pair, an unprimed male, was taken at the same time directly from an all-male holding tank. Four levels of sperm concentration were achieved by mixing the amount of milt estimated to contain 20 µL of packed sperm in 0.5, 1, 2, or 4 L of water. This procedure was replicated with nine females and nine pairs of males.

Immediately before a fertilization trial, a male was stripped of milt and spermatocrit determined. Sperm motility was measured within 1 h of collection. A drop of milt was placed in a 2.0-cm-diameter Plexiglas cell on the stage of a Nikon Diaphot 200 inverted stage microscope fitted with a Cohu Solid State video camera (model 4815, Cohu Inc., San Diego, Calif.). The temperature of the microscope stage was maintained at 10–12°C. The cell was flooded with 0.5 mL water and sperm motility was recorded on a Sanyo video recorder (SRT-500, Sanyo Electric Co. Ltd., Japan). The mean proportion of sperm motile and the duration of motility were assessed from the video record of two or three samples of each specimen of milt.

Nine females were used as egg donors within four days of ovulation. Eggs from a female were divided into batches of 50–60 eggs in plastic petri dishes. An aliquot of milt containing 20 µL of packed sperm was placed on the bottom of a dry plastic container. Water was added by quickly pouring from a height of 30–40 cm. This ensured rapid, complete mixing of the milt. A batch of eggs was added to the milt suspension immediately after the water. After 3–4 min the eggs were transferred to an incubation tray.

Experiment 3: proportion of eggs fertilized following delay after activation of the sperm

An ovulated female was anesthetized and dried, and six batches of eggs (50–100) were stripped into dry 1-L beakers. Aliquots of milt from a male, each calculated to contain 20 µL of packed sperm, were used to fertilize the eggs as follows.

Simultaneous activation of eggs and sperm

Milt was placed out of contact with the eggs and 400 mL of fresh water poured into the beaker. The flow of the water was sufficient to cause a thorough mixing of the gametes.

Delayed insemination of eggs following activation of sperm

Milt was pipetted into 400 mL of water, which was then swirled, and after a delay of 5, 10, 20, 40, or 180 s, the milt suspension was poured onto a batch of eggs. Three to four minutes after insemination, eggs were placed in an incubator. This procedure was repeated for a total of nine male–female pairs.

Experiment 4: proportion of activated eggs fertilized following a delayed insemination

Seven batches of eggs were taken from each of nine females. Aliquots of milt from a male (n = 9), calculated to contain 20 µL of sperm, were added to the eggs as follows.

Simultaneous activation of eggs and sperm

The milt was placed out of contact with a batch of eggs in a dry 1-L beaker and 400 mL of water was poured onto the gametes.

Insemination delayed following activation of eggs

Two hundred millilitres of water was added to a container holding eggs, and after a delay of 5, 10, 20, 40, 80, or 160 s, milt was added by pipette, followed immediately by a further 200 mL of water to complete mixing of the gametes. After 3–4 min, eggs were placed in an incubator tray.

Experiment 5: fertilization rate of eggs immersed for different periods in milt suspension

In each of 10 replicates, six batches of eggs from a female were exposed to sperm from a single male for varying periods of time. In each trial, 500 mL of river water was poured onto an aliquot of milt containing 20 µL of sperm in a 1-L container. Within 3 s, a dry mesh basket containing a batch of 50–100 eggs was dipped into the milt suspension. The eggs were withdrawn from the suspension after 0.5, 2, 5, 10, 20, or 180 s and immediately rinsed in flowing water for 3 min before being placed in an incubator. A video record revealed mean immersion times of 0.48 s ± 0.04 SE (standard error), and 2.05 s ± 0.01 in the two shortest exposure groups.

Statistical procedures

The Friedman two-way analysis of variance (FAOV) was used to test for differences among three or more groups of related samples (Siegel 1956). Wilcoxon's signed-ranks test (WRT) was used to compare paired data, and the Mann–Whitney U test (MWU) was used to compare independent samples. The association between two variables was esti-

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mated as the Spearman rank correlation coefficient (SRC). The level of significance was set at $P = 0.05$.

Results

GSI, milt yield, spermatocrit, and sperm motility of adult and precocious males

GSI was determined in 1998 for 12 precocious males (mean weight 72.9 g ± 1.04) and 19 adult males (230.4 g ± 7.6). The GSIs of adult males (mean = 2.97% ± 0.02) did not differ significantly from those of precocious males (2.94% ± 0.02) (MWU: $U = 110$, $P > 0.05$).

In 1997, the mean yield of milt from 10 adult males (mean weight = 343.9 g ± 23.5) from which all freely running milt was stripped was 1.26 g ± 0.27; the yield from 12 precocious males (78.48 g ± 4.29) was 0.40 g ± 0.15 milt. The difference in milt yield relative to body weight was not significant (MWU: $U = 53$, $P > 0.05$): adult mean yield 0.38% ± 0.09; precocious males 0.46% ± 0.15.

In males from which small milt samples were taken in 1997, spermatocrits of precocious males (mean weight = 85.6 g ± 5.3) increased over the period of sampling (Fig. 1). Differences between the three days of sampling were not significant (FAOV: $\chi_r^2 = 4.6$, $P > 0.05$) although the overall increase from day 0 to day 16 was significant (WRT: $T = 3$, $P < 0.01$). In adults (mean weight = 294.9 g ± 7.6), spermatocrits differed over the period of sampling (FAOV: $\chi_r^2 = 6.9$, $P < 0.05$), declining from day 0 to day 8 (WRT: $T = 6$, $P < 0.05$), followed by a significant increase to day 16 (WRT: $T = 3$, $P < 0.02$). Spermatocrits of precocious males were significantly higher than those of adults on days 8 and 16 (MWU: $U = 16$, $P < 0.02$; $U = 24$, $P < 0.05$).

There were significant changes in the proportion of sperm motile in adult males (FAOV: $\chi_r^2 = 8.15$, $P < 0.02$), showing an increase from day 0 to day 8 (WRT: $T = 0$, $P < 0.01$) (Fig. 2). The proportions of motile sperm in precocious males were not significantly different from those observed in adult males and did not change significantly throughout the period of testing.

There were significant differences in the duration of sperm motility (Fig. 3) over the period of sampling in both adult and precocious males (FAOV: adults, $\chi_r^2 = 17.5$, $P < 0.001$; precocious, $\chi_r^2 = 9.95$, $P < 0.01$). In both groups mean duration rose significantly from day 0 to day 8 (WRT: adults, $T = 0$, $P < 0.01$; precocious males, $T = 2.5$, $P < 0.01$), followed by a decline in adult males (WRT: $T = 1.5$, $P < 0.01$). There were no significant differences between adult and precocious males in duration of motility.

The effect of priming on spermatocrit, sperm motility, and fertilization rate at different sperm concentrations

The amount of milt that could readily be stripped was significantly greater in primed males (MWU: $U = 13$, $P < 0.02$) (Table 1). However, spermatocrits were similar in the two groups. The proportion of motile sperm was greater in the primed males, but the difference was not significant, and there were no significant differences in the duration of motility.

One unprimed male did not fertilize any eggs in any trial. Eggs from the same female were fertilized by sperm from a primed male, suggesting that the unprimed male was infertile, even though sperm were highly motile. Data from the trials with the infertile unprimed male are excluded from further analysis. There were insufficient eggs to complete two trials at the lowest concentration of unprimed male sperm. The proportion of eggs fertilized in the remaining trials ranged from 0% to almost 97%. In both treatment groups, fertilization rate declined with increasing dilution of the milt (Fig. 4). However, there was considerable variation within any one dilution treatment and only in primed males

Fig. 1. Spermatocrit (mean % + standard error) of milt from adult (open bar, $n = 10$) and precocious males (solid bar, $n = 12$) at days 0, 8, and 16 of experiment 1. Asterisk indicates a significant difference between adults and precocious males (MWU: $P < 0.05$). Different letters over the bars indicate significant differences between sample days (WRT: $P < 0.05$). Bars without letters do not differ significantly.

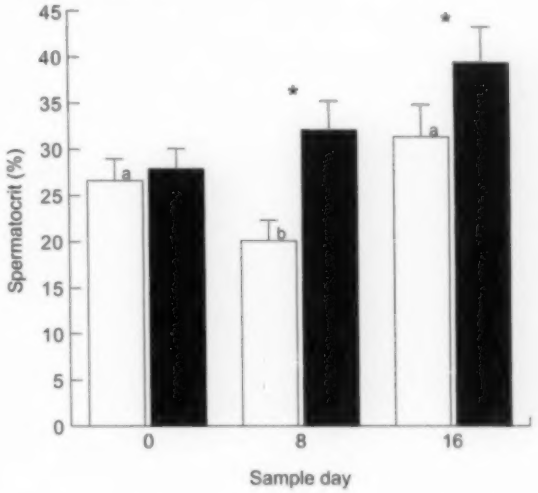

Fig. 2. Mean ranked proportion (+ standard error) of sperm motile 10 s after activation of milt from adult (open bar, $n = 10$) and precocious males (solid bar, $n = 12$) on days 0, 8, and 16 of experiment 1. Different letters over the bars indicate significant differences between sample days (WRT: $P < 0.05$). Bars without letters do not differ significantly.

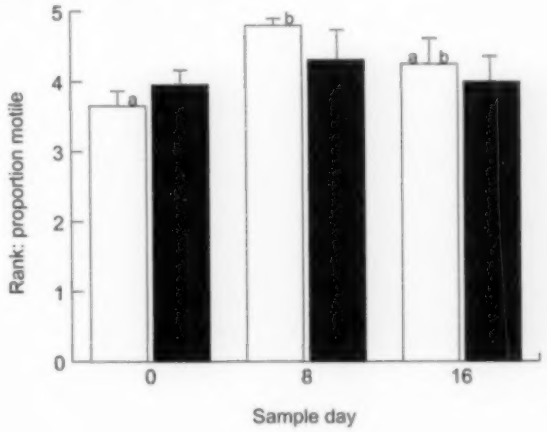

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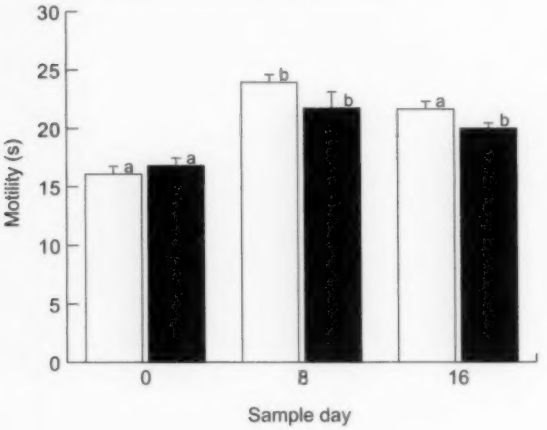
Fig. 3. Duration of motility (mean seconds + standard error) following activation by fresh water of milt from adult (open bar, $n = 10$) and precocious males (solid bar, $n = 12$) on days 0, 8, and 16 of experiment 1. Different letters over the bars indicate significant differences between sample days (WRT: $P < 0.05$). Bars without letters do not differ significantly.



were the differences between concentrations significant (FAOV: $\chi_r^2 = 7.8$, $P < 0.05$) and was the difference between the highest and lowest concentration significant (WRT: $T = 5$, $P < 0.05$). Even at the lowest concentration of sperm, the fertilization rate was 86% in one trial. Differences in the proportions of eggs fertilized by milt from primed and unprimed males were not significant at any milt dilution.

Apart from a positive correlation between the rank of sperm motility and fertilization rate in trials with the highest concentration of primed male sperm (Spearman $\rho = 0.73$, $P = 0.026$), there were no other significant correlations between fertilization rate and either measure of sperm motility of primed and unprimed males. In view of absence of any other correlations, this apparently significant relationship should be considered a type 1 error (Siegel 1956). The ranked proportion of primed male sperm active was correlated with the duration of motility (Spearman $\rho = 0.74$, $P = 0.03$). In unprimed males, the correlation 0.64 was not significant at $P < 0.05$.

Proportion of eggs fertilized after a delay following activation of the sperm, activation of eggs, or after dipping eggs into a milt suspension

The average fertilization rate 0 s, 5 s, or 10 s after activation of the sperm was close to 80% (range 20–99%) and did not differ significantly among the three treatments (Fig. 5). There was a significant decline in the mean fertilization rate when the delay increased from 10 s to 20 s (WRT: $T = 4$, $P < 0.05$), and a sharp drop from 59% to 9% (WRT: $T = 0$, $P < 0.01$) as the delay increased to 40 s. One or two eggs were fertilized in clutches from two of nine females when fertilization was delayed 180 s. There were significant correlations between duration of motility and fertilization rate after delays of 0 s, 5 s, and 20 s (Table 2). The correlation between the proportion of sperm active at 10 s and fertilization rate was low and not significant after any delay.

There were no significant differences in fertilization rate when milt was added 0, 5, 10, and 20 s after activation of the eggs (Fig. 6). There was a sharp, significant decrease in fertilization rate as the delay increased from 20 s to 40 s (WRT: $T = 0$, $P < 0.01$) followed by further declines after 40-s ($T = 0$, $P < 0.01$) and 80-s ($T = 4$, $P < 0.05$) delays. A small number of eggs were fertilized in batches of eggs from eight of nine females after 80 s. Even after a delay of 160 s, small numbers of eggs were fertilized in clutches from seven of nine females. The correlations between sperm motility and the fertilization rate were low and not significant except for that between duration of motility and a delay of 160 s (Table 2).

When eggs were removed from milt suspension and rinsed to remove milt after 0.5 s and 2 s, mean fertilization rates were 13.6% (range 0–27) and 23.5% (3–61), respectively, and did not differ significantly (WRT: $T = 9$, $P > 0.05$) (Fig. 7). Mean fertilization rate increased to over 80% after 5 s (WRT: $T = 1$, $P < 0.01$) and did not increase significantly with longer exposure times.

Discussion

The mean weight of precocious males was less than 33% that of adult males. However, GSIs and milt yields were almost identical. The lack of a difference in relative testis size of precocious and adult male rainbow trout contrasts with findings in Atlantic salmon (Gage et al. 1995), sockeye salmon (Foote et al. 1997), and other teleosts (Taborsky 1998), in which small males adopt a sneaking tactic and have GSIs considerably greater than the larger adult males. Generally, a greater GSI is regarded as an adaptation to enhance the reproductive contribution of a small male adopting a subordinate or sneaking mating tactic.

Spermatocrits were slightly higher in precocious male rainbow trout than in adults and rose over the spawning period. Daye and Glebe (1984) and Hoysak and Liley (2001) reported higher spermatocrits in precocious salmon parr and 3-year male sockeye as compared with anadromous and 5-year males in Atlantic salmon and sockeye, respectively. There are no estimates of the amounts of milt released by competing males during a spawning event; nevertheless, it seems unlikely that the increased concentration of sperm in the milt of precocious males is sufficient to compensate, in number of sperm released at a spawning, for the overall differences in the amount of available milt in the different age classes.

The similarity in the GSIs and milt yield of precocious and adult rainbow trout suggest that in contrast to Atlantic and sockeye salmon, precocious rainbow trout make a gametic investment in reproduction, relative to body size, similar to that of adults. However, whereas no 3-year sockeye salmon (Hoysak and Liley 2001) and few precocious parr of Atlantic salmon (Fleming 1996) survive their first breeding season, most if not all precocious Pennask male trout survive, and presumably, spawn in subsequent years (in the laboratory, precocious males undergo gonadal regression and survive; adults die after spawning; N.R. Liley, unpublished observations). This suggests that in rainbow trout, current reproductive investment by precocious males may be limited

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Table 1. Mean weight (± standard error), milt yield, spermatocrit, and sperm motility of primed and unprimed males.

Males	Weight (g)	Milt (g)	Spermatocrit (%)	Sperm motility	
				Rank	Duration (s)
Primed	240.4 ± 13.0	1.58 ± 0.22	44.33 ± 2.17	3.49 ± 0.48	23.49 ± 1.53
Unprimed	245.7 ± 8.95	0.89 ± 0.12	42.0 ± 4.27	2.73 ± 0.57	23.33 ± 1.31

Fig. 4. Proportion (mean % + standard error) of eggs fertilized by milt from primed (open bars, $n = 9$) and unprimed males (solid bars, $n = 9$) at decreasing concentrations of sperm: 20 µL of packed sperm in 0.5, 1, 2, or 4 L fresh water (experiment 2). Different letters over the bars indicate a significant difference in fertilization rate at different concentrations of sperm (WRT: $P < 0.05$).

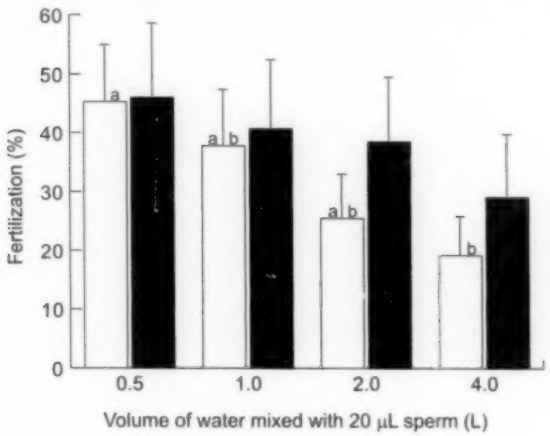
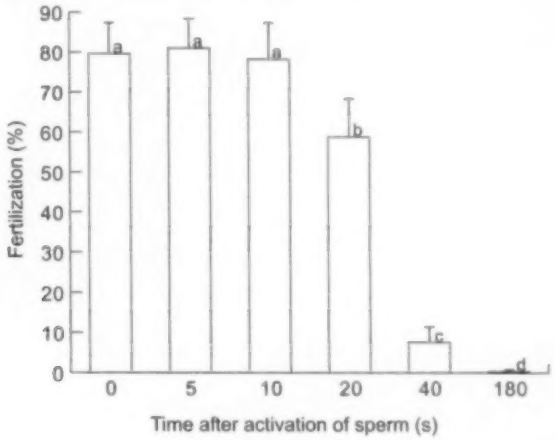


Fig. 5. Proportion (mean % + standard error) of eggs fertilized when milt suspension (20 µL packed sperm in 0.4 L water) was added to eggs concurrently or 5, 10, 20, 40, or 180 s after activation of the sperm by fresh water in experiment 3. Milt from one male ($n = 9$) was used to fertilize six batches of eggs from one female. Different letters over the bars indicate significant differences in fertilization rate at different times after activation of the sperm (WRT: $P < 0.05$).



by the need to conserve resources for future reproductive opportunities.

Following an increase in both measures of motility from day 0 to day 8, there were no further changes in motility over the period of sampling. Furthermore, there were no consistent differences between adult and precocious males. Büyükhatipoglu and Holz (1984) noted an increase in motility of sperm from the beginning to mid-season, followed by a decline over an extended season in farmed fish. They found no difference in the sperm motility of 2- and 3-year rainbow trout, whereas Linhart (1984) reported greater motility in younger male trout. Greater sperm motility in younger age classes has also been reported in Atlantic salmon (Daye and Glebe 1984; Gage et al. 1995) and in sockeye salmon (Hoysak and Liley 2001).

The relationship between sperm motility and fertilizing ability remains equivocal. A positive correlation has been found in hatchery-raised rainbow trout (Moccia and Munkittrick 1987; Ciereszko and Dabrowski 1994), but not in Atlantic salmon (Aas et al. 1991), sockeye salmon (Hoysak and Liley 2001), or Atlantic cod, *Gadus morhua* (Trippel and Neilsen 1992). Experiments in which mixing of the gametes was delayed following activation of the sperm show very clearly that the period of fertility corresponds closely to the duration of sperm motility. The results reported here are in agreement with the findings of other investigations, which indicate a sharp decline in fertilization rate with time from sperm activation (Ginsberg 1963; Hoysak and Liley 2001).

Although not examined systematically, it was clear that small numbers of sperm remained active for 5–20 s after the majority of sperm were no longer motile. It seems likely that these "long-lived" sperm were responsible for the small number of fertilizations 40 s or more after activation. A similar explanation for fertilizations that occurred several minutes after dilution of milt is suggested by Billard (1983) and Ginsberg (1963). In experiments with *Oncorhynchus tshawytscha*, small numbers of eggs were fertilized up to 7.5 min after activation of the sperm (Rutter 1902).

There was no correlation between fertilization rate and the proportion of sperm motile 10 s after activation. This suggests that the amount of milt used in our trials resulted in a sperm density above a threshold level at which the fertilization was restricted by a lack of motile sperm.

The density of sperm used in our trials was, by a subjective assessment of turbidity, similar to that present in a redd at spawning. However, milt is dispersed from a redd over a period of 20–25 s, whereas in our trials the concentration of the milt remains constant. Moccia and Munkittrick (1987) found no correlation between dry fertilization rate and motility in rainbow trout at a high sperm concentration, but at a low sperm concentration the correlation was a highly significant in trials carried out early in the season.

Priming resulted in an increase in the amount of milt and a suggestive, but not significant, increase in proportion of motile sperm. There was no difference in duration of motil-

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Table 2. Correlation (Spearman ρ) between fertilization rate and sperm motility (proportion of sperm motile 10 s after activation and duration of motility) when mixing of the gametes was delayed following activation of the sperm and activation of the eggs.

	Time (s)						
	0	5	10	20	40	80	160
Time between activation of the sperm and mixing with the eggs							
Rank: proportion motile	0.015	0.335	–0.439	–0.132	–0.182	0.118	
Duration of motility	0.749*	0.802**	0.226	0.673*	0.169	–0.089	
Time between activation of the eggs and mixing with milt							
Rank: proportion motile	–0.079	–0.180	0.165	0.347	0.205	0.383	0.012
Duration of motility	0.004	–0.233	–0.112	0.512	0.191	–0.223	0.654*

*$P < 0.05$.
**$P < 0.01$, 1-tailed.

Fig. 6. Proportion (mean % + standard error) of eggs fertilized when milt (20 µL packed sperm) was added concurrently or 5, 10, 20, 40, 80, or 160 s after activation of eggs by 0.4 L fresh water in experiment 4. Milt from one male ($n = 9$) was used to fertilize seven batches of eggs from one female. Different letters over the bars indicate significant differences in fertilization rate at different times after activation of the eggs (WRT: $P < 0.05$).

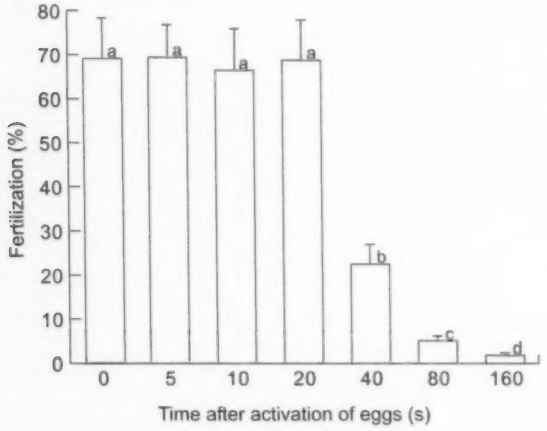
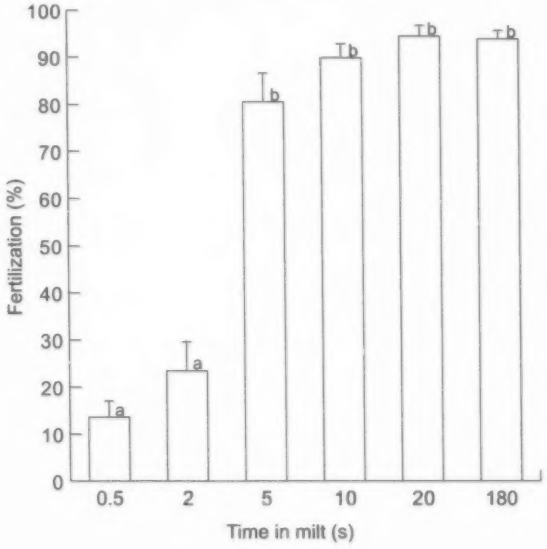


Fig. 7. Proportion (mean % + standard error) of eggs fertilized when eggs were dipped for 0.5, 2, 5, 10, 20, or 180 s in a suspension of milt (20 µL packed sperm in 0.5 L water) in experiment 5. Milt from a single male ($n = 10$) was used to fertilize six batches of eggs from one female. Different letters over the bars indicate significant differences in fertilization rate following different periods of exposure of eggs to milt (WRT: $P < 0.05$).



ity. Until more is known of the amounts of milt released by dominant and subordinate males at spawning, it remains to be determined whether the increase in milt in a primed male results in a greater amount of milt released at spawning and (or) allows a primed male to maintain output over several spawning events.

Fertilization rate declined with increasing dilution of the milt from both primed and unprimed males. However, there was considerable variability in fertilization rate within and between dilutions. The differences between different dilutions and between clutches fertilized by sperm from primed and unprimed males were not significant. Working with a considerably wider range of concentrations of sperm, Hoysak and Liley (2001) obtained a highly significant effect of concentration on fertilization rate in trials with sockeye salmon.

The fertility of eggs decreases after activation by fresh water, but the decline is less rapid than for the sperm. Even though fewer than 5% of eggs were fertile after 80 s, a small number of eggs were fertilized 160 s after activation. An initial sharp decline in fertilization rate, followed by the persistence of fertility in small numbers of eggs 3 or 4 min after activation was also found in *O. tshawytscha* (Rutter 1902) and *O. nerka* (Hoysak and Liley 2001).

In salmonids, fertilization is normally initiated when one sperm enters the micropyle and fuses with the oocyte plasma membrane (Ginsberg 1963; Szollosi and Billard 1974; Kobayashi and Yamamoto 1981). Yanagimachi et al. (1992) found that sperm orient towards the micropyle only when they are in contact or near contact with the egg. Our experiments indicate that some form of attachment to as many as 27% of a batch of eggs may take place within 0.5 s, and up to 61% are fertilized after only 2 s. Five seconds immersion in milt suspension was sufficient for over 90% of the eggs to be fertilized. It is not possible from this study to determine whether this attachment is the result of entry into the micropyle, or to what extent sperm are retained in the surface film of water around the egg. Electron microscopy studies of chum salmon eggs (*Oncorhynchus keta*) by Kobayashi and

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Yamamoto (1981) show that within 15 s a single "fertilizing" sperm has already passed through the micropyle and entered the ooplasm, indicating that initial entry into the micropyle occurs within seconds of mixing of the gametes. In the medaka (*Oryzias latipes*), the mean time required for the sperm to attach to the egg surface through the micropyle was 3 s, with a range of 1–6 s (Iwamatsu et al. 1991). The presence of a sperm lodged in the micropyle is believed to prevent the entry of other sperm (Szollosi and Billard 1974; Kobayashi and Yamamoto 1981; Yanagimachi et al. 1992).

The short duration of sperm motility of salmonids is in sharp contrast to the extended motility of sperm in some other teleosts (Billard 1986; Stockley et al. 1997). The short duration of motility and egg viability, rapid entry of the sperm, and subsequent blockage to polyspermy may contribute to the reproductive success of a dominant male in competition with other males. Even though a spawning group may be surrounded by a cloud of milt, only sperm released close to the eggs are likely to encounter unfertilized eggs within the duration of gamete viability. There is no wide dispersal of the eggs comparable to that of the sperm. Eggs are dense and fall rapidly between the larger pieces of gravel that line the bottom of the nest over an area of 5–10 cm diameter. The dominant male initiates spawning, and during emission of the gametes, the dominant male's vent is close to that of the female. Other males that arrive within the next 1–2 s are unlikely to achieve a position as close to the female. The motility of the sperm is unlikely to significantly reduce the distance disadvantage of subordinate males. Billard and Cosson (1992) estimate that sperm released on the side of an egg opposite to the micropyle are unable to reach the micropyle within the active life of the sperm. Finally, although turbulence of the water will undoubtedly mix gametes from several males with those of the female, our observation of the movement of milt indicates that turbulence is much reduced within the nest.

The contribution of close proximity to the success of a dominant male may be enhanced by the influence of a "maternal factor" (Litvak and Trippel 1998). It is well established in salmonids and other teleosts (Scott and Baynes 1980; Amanze 1994; Litvak and Trippel 1998) that the intensity of movement and the proportion of sperm active is greater in coelomic fluids than in fresh water alone. Scott and Baynes (1980) conclude that the activating effect of coelomic fluid in salmonids is attributable to the ion balance in the fluid rather than the presence of a specific "gamone". On the other hand, Amanze (1994) reported directional movement towards the source of ovarian–follicular fluid by the sperm of the rosy barb, *Barbus conchonius*, and provides evidence of a species-specific soluble factor in the ovarian fluids affecting sperm motility in that species.

These findings about the effect of coelomic fluid on sperm motility suggest that, because dominant males are able to release their gametes close to the eggs at oviposition, the sperm of the dominant male may be exposed to a higher concentration of ovarian fluids released with the eggs than sperm of subordinate males. An increase in sperm motility triggered by coelomic fluid may enhance the competitive advantage of the dominant male's sperm over those of males releasing their gametes slightly later or at a greater distance from the female.

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PERSPECTIVE

Synthesis of the toxicological impacts of the *Exxon Valdez* oil spill on Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, U.S.A.

M.G. Carls, G.D. Marty, and J.E. Hose

Abstract: Pacific herring (*Clupea pallasi*) in Prince William Sound (PWS) were affected by two major events in the past decade: the *Exxon Valdez* oil spill in 1989 and a 75% collapse in the adult population in 1993. In this review we compare and reinterpret published data from industry and government sources. Combining site-specific estimates of exposure and recent laboratory effects thresholds, 0.4–0.7 $\mu g \cdot L^{-1}$ total polynuclear aromatic hydrocarbons, we conclude that 25–32% of the embryos were damaged in PWS in 1989. Significant effects extended beyond those predicted by visual observation of oiling and by toxicity information available in 1989. Oil-induced mortality probably reduced recruitment of the 1989 year class into the fishery, but was impossible to quantify because recruitment was generally low in other Alaskan herring stocks. Significant adult mortality was not observed in 1989; biomass remained high through 1992 but declined precipitously in winter 1992–1993. The collapse was likely caused by high population size, disease, and suboptimal nutrition, but indirect links to the spill cannot be ruled out. These concepts have broad application to future oil spill assessments. For example, safety standards for dissolved aromatics should reflect the previously unrecognized high toxicity of polynuclear aromatic hydrocarbons to adequately protect critical life stages.

Résumé : Deux événements majeurs, le déversement de mazout de l'*Exxon Valdez* en 1989 et l'effondrement de l'ordre de 75 % de la population adulte en 1993, ont affecté les Harengs du Pacifique (*Clupea pallasi*) du détroit du Prince-William (PWS). On trouvera ici une comparaison et une ré-interprétation des données déjà publiées sur le sujet par l'industrie pétrolière et les gouvernements. La combinaison des estimations des expositions aux divers sites et la détermination récente en laboratoire des seuils à partir desquels les effets des hydrocarbures aromatiques polynucléaires (PAH) se font sentir, soit 0,4–0,7 $\mu g \cdot L^{-1}$, nous amènent à conclure que 25–32 % des embryons du PWS ont été endommagés en 1989. Les effets significatifs ont dépassé ceux qu'on pouvait prédire à l'observation visuelle du déversement de mazout et à l'aide des données toxicologiques disponibles en 1989. La mortalité due au mazout a sans doute réduit l'apport de la classe d'âge de 1989 à la population, mais il est impossible de quantifier cette réduction, parce que le recrutement a aussi été faible chez les autres stocks de harengs de l'Alaska. Aucune mortalité significative n'a été observée chez les adultes en 1989; la biomasse est demeurée élevée jusqu'à la fin de 1992 pour décliner très rapidement durant l'hiver 1992–1993. L'effondrement a été sans doute causé par une densité élevée de la population, par la maladie et par une alimentation suboptimale; on ne peut cependant pas éliminer les effets indirects du déversement de mazout. Ces concepts pourront, en grande partie, s'appliquer à l'évaluation de déversements pétroliers futurs. Par exemple, les normes de sécurité pour les substances aromatiques dissoutes devront tenir compte de la forte toxicité des PAH, inconnue encore récemment, si on veut protéger adéquatement les stades critiques des cycles biologiques.

[Traduit par la Rédaction]

Introduction

A decade after the *Exxon Valdez* spilled roughly 42 million litres of Alaska North Slope crude oil (ANSCO) into Prince William Sound (PWS), Alaska, controversy continues about the extent and duration of the impact on Pacific herring (*Clupea pallasi*). Industry investigators (Bienert and Pearson 1995; Pearson et al. 1995, 1999) consistently concluded that

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M.G. Carls.[1] National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801, U.S.A.
G.D. Marty. Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA 95616-8732, U.S.A.
J.E. Hose. Occidental College, Los Angeles, CA 93420, U.S.A.

[1]Corresponding author (e-mail: mark.carls@noaa.gov).

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Fig. 1. Mean total polynuclear aromatic hydrocarbon (TPAH) concentration in (*a–b*) seawater, (*c*) mussel tissue, and (*d*) sediment, as functions of time (*a*) throughout Prince William Sound (Neff and Stubblefield 1995) and (*b–d*) at specific locations within the Naked Island area (Short et al. 1996). Where mussel and sediment symbols are solid, *Exxon Valdez* oil (EVO) was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). (Outside Bay mussels (only) were analyzed with two PAH identification models; EVO was confirmed by both models where symbols are solid; half-filled symbols indicate additional samples where only the Bence and Burns (1995) model identified EVO.) Solid symbols for seawater data indicate that one or more samples contained naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes; (*a*) composition data from Neff and Stubblefield (1995) were not available. Lines labeled bg are estimated background concentrations. Timing of herring spawn and estimated hatch times (assuming a 24-day incubation time) are indicated. The T/V *Exxon Valdez* spilled oil March 24, 1989.

oil effects in 1989 were of smaller magnitude, spatial, and temporal extent than did Natural Resource Damage Assessment (NRDA) investigators (e.g., Brown et al. 1996*a*; Hose et al. 1996; Marty et al. 1997). Reconciliation of NRDA and industry data has been complicated by conflicting methods for detecting *Exxon Valdez* oil (EVO) in the environment, differing estimates of the magnitude of oil exposure, mechanisms of potential toxicity, and difficulties in interpreting population data. By 1990, the preponderance of data from both groups suggested that residual oil impacts were undetectable in embryos and larvae. Stock size in PWS was at near-record levels when the spill occurred, remained strong until 1992, and did not appear to be affected by the oil spill (John Wilcock, Alaska Department of Fish and Game, Cordova, Alaska, personal communication). However, the PWS herring population collapsed over the winter of 1992–1993, closing the multimillion-dollar commercial fishery and raising concerns that the collapse was related to the spill.

The main purpose of this review is to examine evidence for short-term consequences of the EVO spill on all life stages of Pacific herring in an effort to reconcile divergent NRDA and industry conclusions. Exhaustive review of the hydrocarbon literature and other oil spills is beyond the scope of this paper; instead, references are limited to those describing weathered oil or specific constituents of EVO or ANSCO. Spatial and temporal distributions of oil in PWS in 1989 are considered key to understanding the magnitude and mechanisms of exposure and subsequent short-term effects. We performed a number of new data analyses comparing methods to detect weathering oil in organisms, coupling oil measurements in the environment and in organisms, validating the use of resident mussels as indicators of oil exposure for herring eggs, and refining the estimates of toxic total polynuclear aromatic hydrocarbon (TPAH) concentrations, and thus the extent of biologically relevant exposure in PWS in 1989.

A second objective is to discuss the possibility of long-term spill consequences raised by the collapse of the herring population in 1993. Because our findings generally paralleled those of industry, we provide only a limited discussion of this topic; interested readers should refer to Pearson et al. (1999) and Carls et al. (2001*a*) for further detail. Pearson et al. (1999) conclude increases in herring biomass and decreased food supply, in combination with disease or other natural factors, led to the collapse. Carls et al. (2001*a*) agree, but do not rule out indirect links to the spill.

Petroleum hydrocarbon measurement, identification, and weathering

Not only do industry and NRDA researchers reach many conflicting conclusions regarding the impacts of the EVO spill, they also developed different analytical tools to reach these conclusions. Consequently, we begin with a brief review of oil identification methods. These different methods have, in part, contributed to the differing conclusions reached by the two groups.

Oil identification models independently developed by industry researchers (Bence and Burns 1995) and NRDA researchers (Short and Heintz 1997) successfully recognize pure and weathered EVO in sediment and mussels (Fig. 1), but identification is complicated by changes in polynuclear aromatic hydrocarbon (PAH) composition in water and other tissue, such as herring eggs, as a result of differential hydrocarbon accumulation and (or) metabolism. Both models successfully detected EVO in contaminated mussels, which have little ability to metabolize hydrocarbons (Vandermeulen and Penrose 1978; Stegeman 1985; Livingstone et al. 1989). For example, the Short and Heintz model detected EVO in all analyzable Outside Bay mussel samples in 1989, and the Bence and Burns model found EVO in 36 of the 46 samples (78%) (Fig. 1). The differences between PAH composition in nonmolluscan tissue and source oil that were observed in eggs and adult herring tissues experimentally exposed to ANSCO (Carls et al. 1998, 1999, 2000) were anticipated by Bence and Burns (1995), who stated that their model was most applicable to samples of external surfaces and gastrointestinal tracts and that shifts in PAH composition in internal tissues lead to uncertainties in identifying EVO in tissue. Similarly, data from the same laboratory tests could not be analyzed with the Short and Heintz model because some PAH essential for identification were absent. The Bence and Burns model correctly identified EVO in only 4% of these eggs and did not identify EVO in herring muscle tissue or ova, thus identification of contaminant hydrocarbons was usually erroneous because a source of oil is always assigned. Differences between PAH composition in water and source oil also cause difficulties in identification of the source oil because dissolution rates decline as the number of aromatic rings increases (Page et al. 1995; Short and Heintz 1997). Thus, composition of PAH in organisms contaminated by exposure to dissolved PAH may frequently not resemble composition in the source oil for multiple reasons. Because the Short and Heintz (1997) model is less likely to misidentify the source of contaminant oil, we prefer it and use it where applicable throughout the remainder of this paper.

Oil weathering also alters PAH composition. Although the rate at which oil weathers in PWS is extremely variable (the weathering index (w) of oil in beach sediment ranged from 0 to 10.7 in 1995 (Carls et al. 2001*b*)), the close correspondence between PAH composition in the field and laboratory demonstrates that the weathering processes are fundamen-

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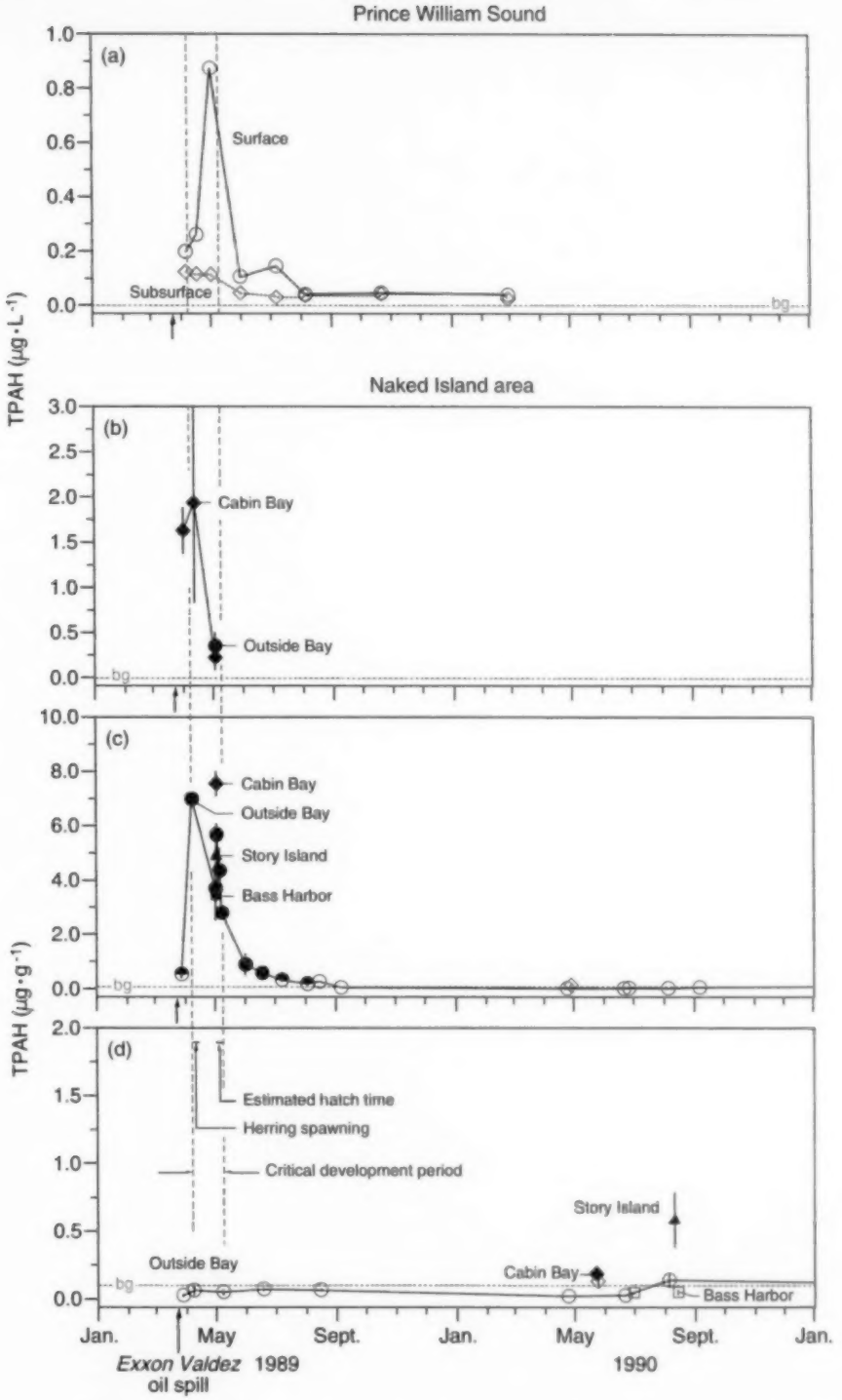

tally identical (Short and Heintz 1997). Carls et al. (2001*b*) provide the following verbal descriptions for w: unweathered, $w = 0$; slightly weathered, $0 < w \leq 2$; moderately weathered, $2 < w \leq 8$; and highly weathered, $w > 8$. Whereas the weathering model developed by Short and Heintz (1997) does not explicitly address the weathering of other petroleum hydrocarbons, e.g., alkanes and the unresolved complex mixture (UCM), there is no evidence at present to

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suggest that changes in concentration and composition of these compounds in laboratory tests would be substantively different from changes in the field.

Short-term spill consequences

Hydrocarbon data from industry and NRDA sources were reexamined to determine the temporal and spatial extent of EVO in areas where Pacific herring spawned in 1989. Data available and appropriate for assessment of site contamination include visual observations (e.g., Gundlach et al. 1990; Neff et al. 1995) and chemical analyses of seawater (Neff and Stubblefield 1995; Short and Harris 1996*a*), sediment (O'Clair et al. 1996; Short and Babcock 1996), molluscs (e.g., Brown et al. 1996*a*; Short and Babcock 1996), and herring eggs (Pearson et al. 1995; Short et al. 1996). TPAH concentrations in seawater, sediment, and mussels from NRDA studies were obtained directly from the NRDA hydrocarbon database (Short et al. 1996). Published data, including text and graphics from industry-sponsored research were included as appropriate, but detailed data from industry studies were generally not available. Sites where herring spawned that were nominally classified as oiled (the Naked Island area and northern Montague Island) were examined most closely. There was no argument between industry and NRDA that sites in the northern and northeastern portions of PWS were not oiled (e.g., Fairmont Island and Tatitlek Narrows), and these sites were used in NRDA studies as EVO-free reference areas.

Spatial distributions of oil within spawning areas

Pacific herring spawn biomass and distances spawned in PWS were estimated by the Alaska Department of Fish and Game (Brady et al. 1991), and both industry and NRDA researchers accepted these estimates (Bienert and Pearson 1995; Brown et al. 1996*a*, 1996*b*). Of the total spawned biomass, 32% was in the northern Montague Island area, 19% was in the Naked Island area, and the remainder was in the northern and northeastern areas (Brady et al. 1991). Distances of shoreline with spawn in oiled areas were relatively smaller, 29% and 14% of total spawn length, for the Montague Island and Naked Island areas, respectively (Brady et al. 1991). Industry estimates of potentially oiled herring spawn were based on spawn distance (Pearson et al. 1995), whereas NRDA estimates were based on spawn biomass (Brown et al. 1996*a*, 1996*b*).

Designation of potentially oiled spawn areas by NRDA and industry were identical (Pearson et al. 1995; Brown et al. 1996*a*). The Naked Island area and northern Montague Island were both within the observed distribution of oil (Gundlach et al. 1990) (Figs. 2–3). Oiling was documented along the northern shoreline of Montague Island, including areas in Zaikof Bay, Rocky Bay, Montague Point, Graveyard Point, and Stockdale Harbor (Brady et al. 1991). Islands moderately or heavily impacted by oil included Naked, Storey, and Ingot islands (Brady et al. 1991). In agreement with Brown et al. (1996*a*, 1996*b*), Bienert and Pearson (1995) found 43% of the total miles of spawn were deposited within the slick trajectory, but based on a visual shoreline survey of oiling by Neff et al. (1995), Pearson et al. (1995) concluded that only 4% of the total spawn distance was at risk of coming into direct physical contact with oil.

Biological availability of oil at spawning sites in 1989

EVO was identified as the source of contamination in 1989 in herring spawning sites throughout the Naked Island and Montague Island areas, except for Zaikof Bay, where insufficient data were available (Figs. 2–3). In the Naked Island area, EVO was confirmed in mussel samples from Cabin Bay, Outside Bay, Bass Harbor, on the north shore of Storey Island, and on a small islet to the south of Storey Island (Short and Harris 1996*b*; Short and Heintz 1997). Mussels tethered 25 m below the water surface in Outside Bay were contaminated with EVO (Short and Harris 1996*b*). On Montague Island, EVO was confirmed in mussel tissue from Rocky Bay and Stockdale Harbor and in *Littorina* samples near Graveyard Point. Water samples provided additional evidence of EVO in Cabin Bay and Rocky Bay. In contrast, EVO was rarely identified in sediments collected from these spawning areas, and TPAH concentrations were only slightly elevated (<0.5 $\mu g \cdot g^{-1}$) if at all (Figs. 1 and 4). Thus, EVO was identified in samples from most Pacific herring spawn sites (Short and Heintz 1997) within the slick trajectory (Gundlach et al. 1990), providing justification for designating individual sites within the trajectory as oiled.

Although there was little overlap between visibly oiled shoreline and herring spawn, there was strong evidence of biologically available oil in spawn areas in 1989 (Figs. 2–3). Industry estimates that 4% of the spawn length occurred along visibly oiled shorelines (Pearson et al. 1995) are similar to our estimate (6%). However, widespread contamination of mussels throughout much of the spawn length provides evidence that EVO was biologically available in areas without visible shoreline oiling, as do elevated TPAH concentrations in herring eggs-on-kelp (Pearson et al. 1995). For example, mean TPAH concentrations in mussels at the head of Outside Bay rose to a maximum of 7.0 $\mu g \cdot g^{-1}$ dry weight at a distance of 0.6–1.0 km from the nearest small (<0.1 km) patch of visible oil identified in shoreline surveys. TPAH concentrations in intertidal sediment at the head of Outside Bay remained ≤0.2 $\mu g \cdot g^{-1}$, providing further evidence of minimal sediment contamination where oil was biologically available. Mussels tethered 0.5 km offshore from the nearest oiled shoreline in Outside Bay at depths of 1–25 m accumulated EVO (mean TPAH concentrations ranged from 0.6 to 1.5 $\mu \cdot g^{-1}$ dry weight in May 1989) (Short and Harris 1996*b*), demonstrating that dispersed oil was biologically available in the water column. Thus, oil was biologically available over a much broader range than suggested by visibly oiled shorelines or nearby sediment contamination.

Temporal changes in PAH

Seawater

TPAH concentrations in seawater peaked in the Naked Island (1.9 $\mu g \cdot L^{-1}$) and Montague Island (2.6 $\mu g \cdot L^{-1}$) areas soon after the EVO spill, which was shortly before or at the time Pacific herring spawned in these areas (Brown et al. 1996*a*; Short and Harris 1996*a*; Figs. 1 and 4). Total PAH concentrations in surface water, averaged throughout PWS, peaked in late April (Neff and Stubblefield 1995). Seawater samples at peak concentrations in Rocky Bay and all water samples in the Naked Island area contained naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes at concen-

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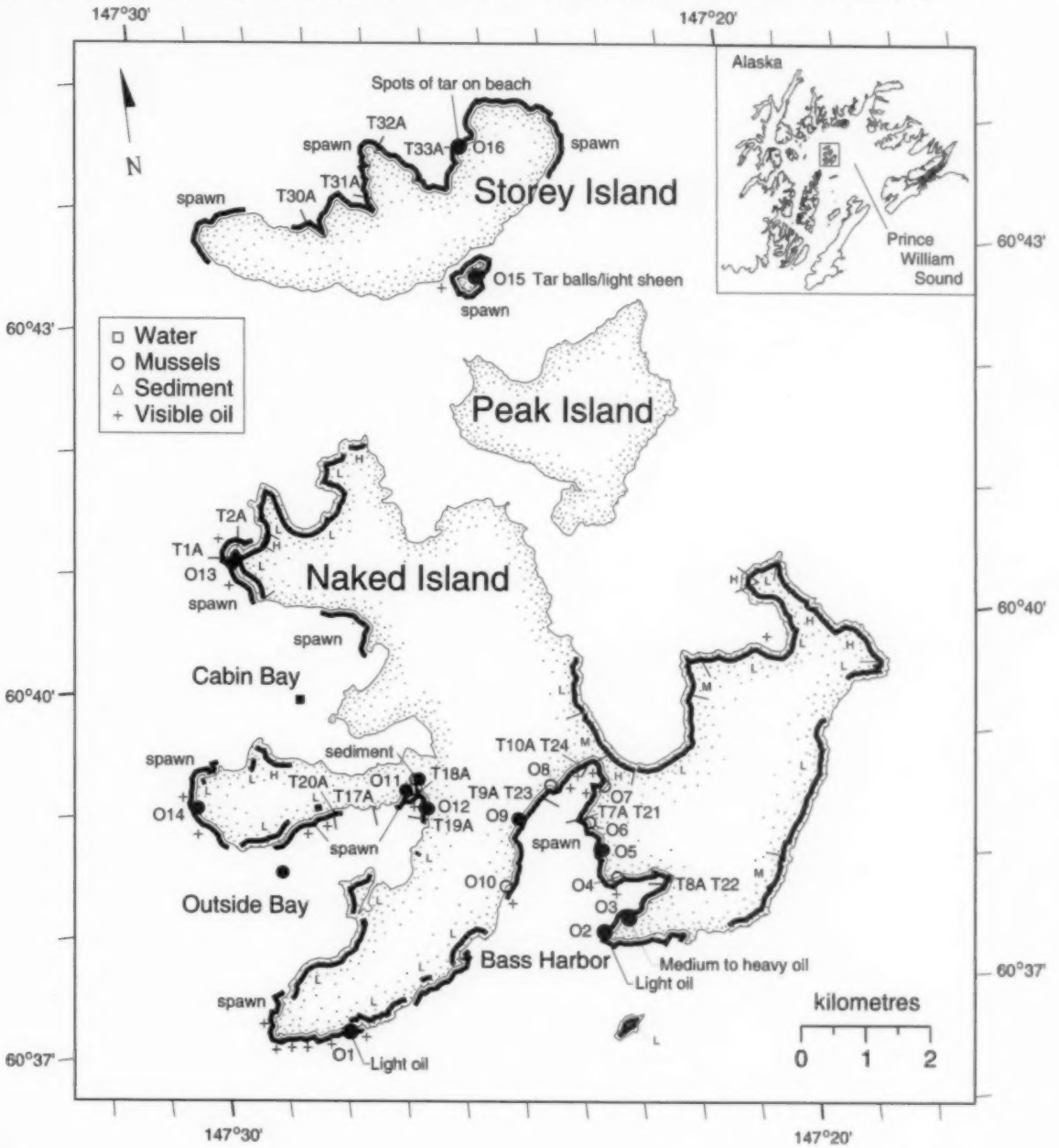
Fig. 2. Location of herring spawn and water, mussel, and sediment hydrocarbon collection sites in 1989 in the Naked Island area. The mapped area was entirely within the *Exxon Valdez* oil (EVO) slick trajectory (Gundlach et al. 1990). Herring spawn data (bold lines drawn slightly offshore) are from Brady et al. (1991). Where symbols are solid, EVO was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Sites labeled O1–O16 were sampled by Brown et al. (1996*a*), T1A–T33A by Pearson et al. (1995), and unlabeled sites represent a mixture of other studies (Short et al. 1996). Visibly oiled samples are annotated (e.g., medium oil), and + mark approximate positions of oil observed during herring spawn surveys (Brown et al. 1996*b*). Visible shoreline oiling (thick lines drawn slightly onshore) was categorized as light (L), medium (M), or heavy (H) (Gundlach et al. 1990; Neff et al. 1995).



trations above the method detection limit, strongly suggesting the source of contamination was EVO. The rapid increase in aqueous TPAH concentrations after the spill, followed by a decline, is unambiguous evidence for a single-event spill, and also suggests that EVO was the source of contamination. Peak TPAH concentrations in water occurred about the

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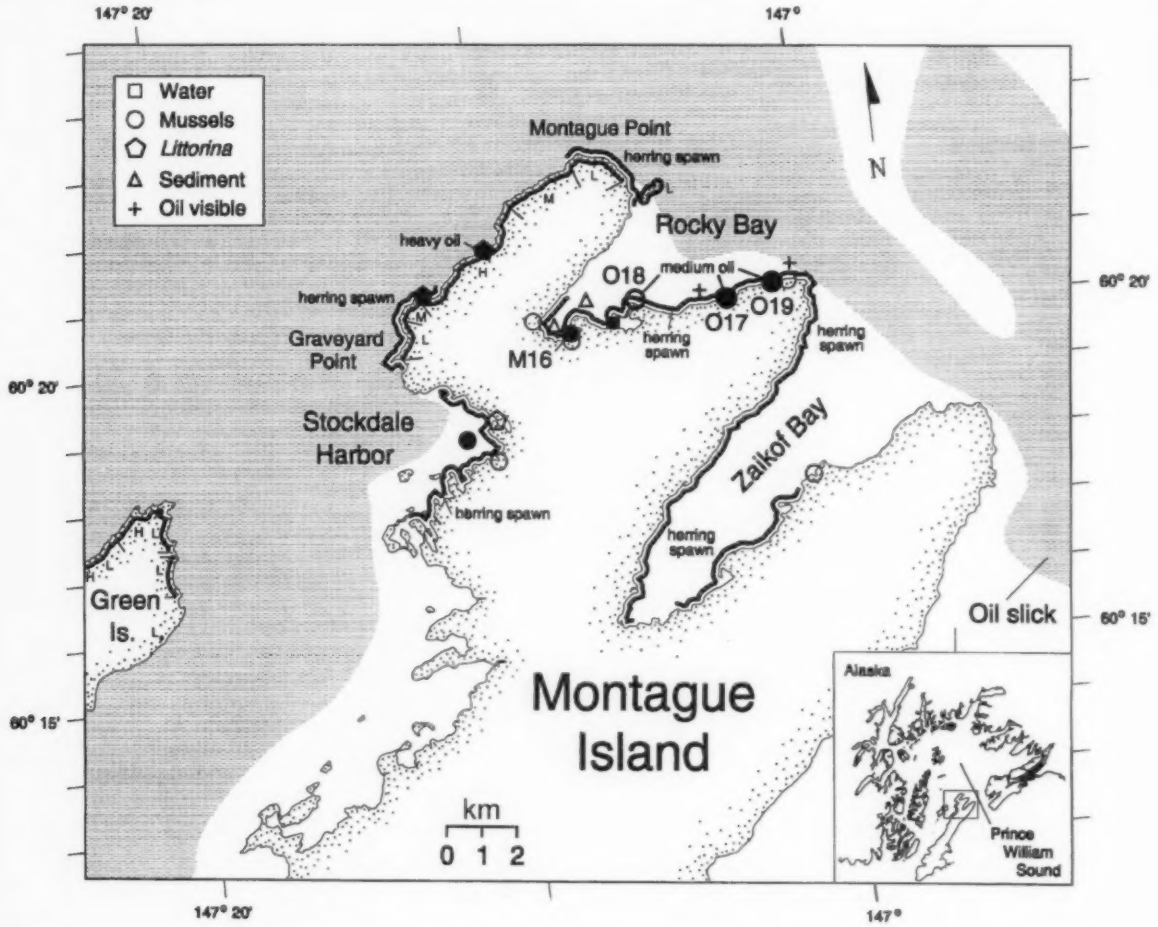
Fig. 3. Location of herring spawn and water, mussel, *Littorina*, and sediment hydrocarbon collection sites in 1989 on northern Montague Island. Herring spawn data (bold lines drawn slightly offshore) are from Brady et al. (1991). Where symbols are solid, *Exxon Valdez* oil (EVO) was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Sites sampled by Brown et al. (1996*a*) for mussels and herring are labeled as originally designated by the authors (O17–O19); unlabeled sites and site M16 represent a mixture of studies (Short et al. 1996). Visibly oiled samples are annotated (e.g., medium oil), and + mark approximate positions of oil observed during herring spawn surveys (Brown et al. 1996*b*). Visible shoreline oiling (lines drawn slightly onshore) was categorized as light (L), medium (M), or heavy (H) (Gundlach et al. 1990; Neff et al. 1995). Oil slick data (crosshatch) are from Gundlach et al. (1990).



same time as peak concentrations in mussels in the Naked Island area (Fig. 1), but earlier than in mussels farther south at Rocky Bay (Fig. 4).

Sediment

EVO was infrequently detected in intertidal sediment from spawn areas within the oil trajectory, and TPAH concentrations were generally low throughout 1989 (Figs. 1 and 4). In 1989, TPAH concentrations were not elevated in sediment at the head of Outside Bay ($\leq 0.2\ \mu g \cdot g^{-1}$), but concentrations at Rocky Bay were slightly greater than estimated background concentrations ($<0.5\ \mu g \cdot g^{-1}$) and remained elevated until mid-1991 (Short and Babcock 1996). However, divers observed oil in sediment in 1989 at Outside Bay, Bass Harbor, Cabin Bay, and Montague Point to Graveyard Point (Pearson et al. 1995), but intertidal sediments were often not collected until 1990. EVO was detected in sediment in 1990 at two of these sites (Cabin Bay and Storey Island; Short and Heintz 1997). Although the extent of sediment sampling in spawn areas was very limited, these data generally corroborate visible evidence of shoreline oiling in 1989 (Gundlach et al. 1990; Neff et al. 1995) or absence thereof.

Mussels

Mussel samples provided strong evidence that oil was biologically available in herring spawn areas within or adjacent to the slick trajectory in 1989 (Figs. 1 and 4). Total PAH concentrations in mussel tissue increased after the spill, peaked at about the time Pacific herring spawned in the Naked Island area, and peaked at about the time of embryo

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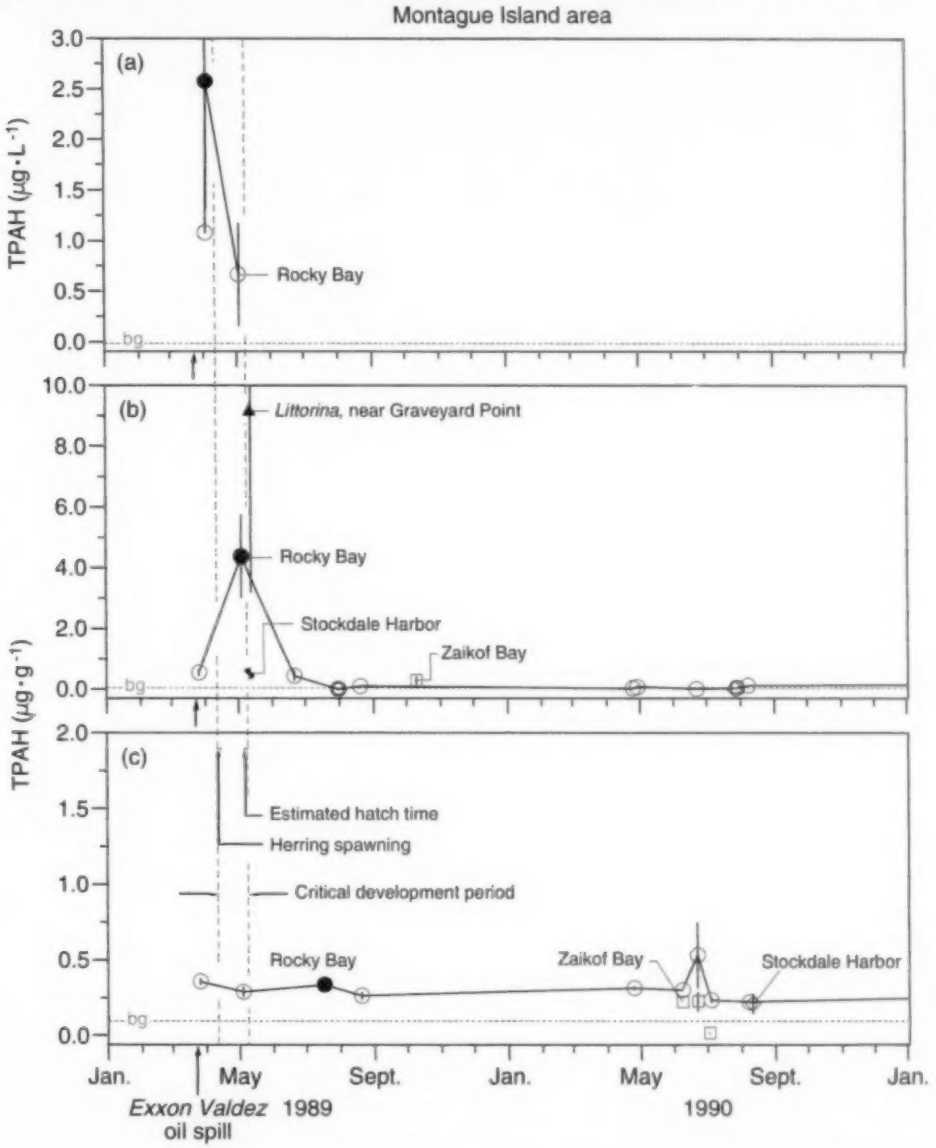
Fig. 4. Mean total polynuclear aromatic hydrocarbon (TPAH) concentration in (*a*) seawater, (*b*) mussel tissue, and (*c*) sediment as functions of time in Rocky Bay, Stockdale Harbor, and Zaikof Bay. Also included are TPAH concentrations in *Littorina* spp. near Graveyard Point. Where mussel and sediment symbols are solid, *Exxon Valdez* oil (EVO) was confirmed as the source of contamination in one or more samples (Short and Heintz 1997). Solid symbols for seawater data indicate that one or more samples contained naphthalenes, fluorenes, dibenzothiophenes, and phenanthrenes. Lines labeled bg are estimated background concentrations. Timing of herring spawn and estimated hatch times (assuming a 24-day incubation time) are indicated. The T/V *Exxon Valdez* spilled oil March 24, 1989.



hatch in the Montague Island area. The complete time series data for Outside Bay and Rocky Bay provide a frame of reference against which other mussel samples could be judged in the these areas. At Outside Bay, TPAH concentrations in mussels increased before herring spawned, were high during spawning (>6.1 $\mu g \cdot g^{-1}$ dry weight), and remained elevated through hatch (>2.5 $\mu g \cdot g^{-1}$) (Fig. 1). Total PAH concentrations in mussels at all other Naked Island sites were similarly high at about the time of peak concentration in Outside Bay (3.4–7.5 $\mu g \cdot g^{-1}$) (Fig. 1). Total PAH concentration in mussels collected near the Ingot Island herring spawn, a site included in the Naked Island area, ranged from 9.9 to 15.4 $\mu g \cdot g^{-1}$ in September 1989, suggesting high exposures in the spring. (Both industry (Pearson et al. 1995) and NRDA (Brown et al. 1996*a*) agree that the Ingot Island area was moderately to heavily oiled.) At Rocky Bay, TPAH concen-

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trations in mussels increased during spawning and peaked at 4.4 $\mu g \cdot g^{-1}$ at about the time of embryo hatch (Fig. 4). Around the time of peak concentration in Rocky Bay, TPAH was high (9.2 $\mu g \cdot g^{-1}$) in *Littorina* spp. near Graveyard Point, but only slightly elevated (0.5 $\mu g \cdot g^{-1}$) in mussels at Stockdale Harbor. Mussel collection in Zaikof Bay began 31 July 1989, well after expected concentration peaks.

Weathering

Increases in oil weathering (w), estimated from mussel tissue, were roughly linear in the first three months ($P < 0.001$), but correlation between w and time was low ($r^2 = 0.28$; Fig. 5). Weathering (w) of EVO ranged from 0.7 to 5.7 in herring-spawn areas during the critical egg incubation period and averaged 3.7 (note: w is a unitless number).

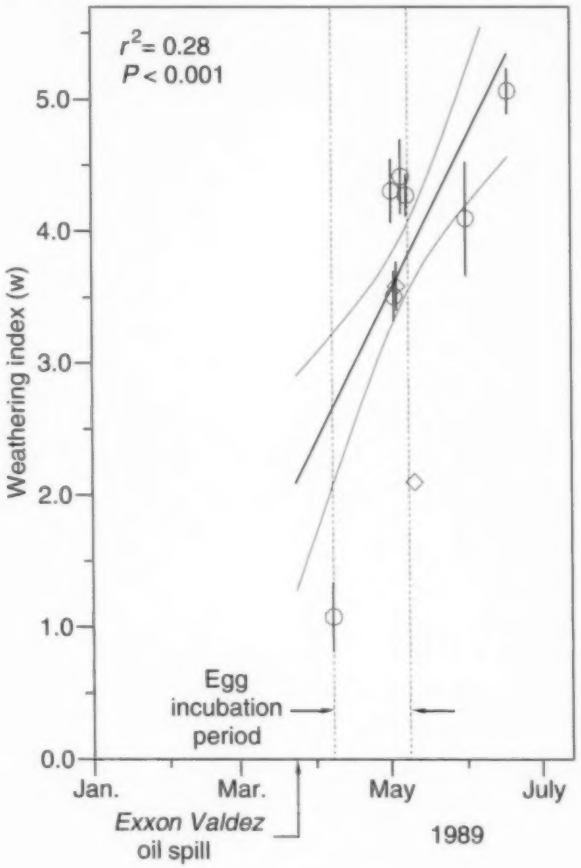
Exposure of herring eggs to oil

Pearson et al. (1995) concluded that mean TPAH concentrations in eggs-on-kelp did not differ significantly between oiled and reference areas in 1989, despite obvious differences between many of the oiled sites and the reference sites (Fig. 6). Mean TPAH concentrations by tide elevation ranged from approximately 27 to 69 $ng \cdot g^{-1}$ at reference sites and 13 to 342 $ng \cdot g^{-1}$ wet weight at oiled sites; maximum concentrations ranged up to 1000 $ng \cdot g^{-1}$ at Cabin Bay, where some eggs were visibly coated by oil (Pearson et al. 1995). The highest mean TPAH concentrations were consistently in the upper tide zone (Pearson et al. 1995), but correlation of TPAH among tide zones was high ($r^2 = 0.93$), demonstrating that surface and subsurface oil concentrations were related (our analysis). Less than 4% of the eggs, all from Cabin Bay, were visibly coated with oil, and the proportion of developed eggs was inversely related to concentration at this site. Pearson et al. (1995) concluded that only a minor portion of the 1989 spawn, the 2% of the spawn length at Cabin Bay, was affected by the spill.

Herring eggs collected by Brown et al. (1996*a*) were analyzed for hydrocarbons, but detection problems limited the usefulness of this collection and the results were not published. Total PAH concentrations in herring eggs collected by Brown's group were significantly elevated at Cabin Bay ($P_{ANOVA} < 0.001$), but not at other oiled sites (our analysis). Brown et al. (1996*a*) concluded that the mass of eggs sampled was insufficient for adequate detection of hydrocarbons, and thus relied on hydrocarbon concentrations in mussels collected within herring spawn areas as surrogate measures of exposure.

The source of PAH in herring eggs within the slick trajectory in PWS in 1989 was probably EVO because seawater, sediment, and mussels were all contaminated with EVO in these areas. We cannot directly test the hypothesis that the herring eggs-on-kelp samples collected by Pearson et al. (1995) were contaminated with EVO because the data were unavailable. Pearson et al. (1995) reported that EVO was identified in only three eggs-on-kelp samples, based on the Bence and Burns (1995) model, but this model failed to correctly identify ANSCO as the source of contamination in herring eggs exposed to this oil in laboratory tests by Carls et al. (1999, 2000). Moderately weathered EVO was identified as the source of contamination in a NRDA herring egg sample from Cabin Bay ($w = 2.3$; Short and Heintz 1997).

Fig. 5. Estimated state of oil weathering at herring spawn sites in Prince William Sound in 1989. Weathering (w) was estimated from polynuclear aromatic hydrocarbon (PAH) composition in mussel tissue collected from oiled areas by Brown et al. (1996*a*) with the methods of Short and Heintz (1997). Circles represent data from the Naked Island area; diamonds represent data from the Rocky Bay area on Montague Island. Timing of the herring egg incubation period is indicated. The T/V *Exxon Valdez* spilled oil March 24, 1989.



Exposure of mussels and eggs to oil in spawn areas was water mediated

We infer that EVO was biologically available primarily from the water column in herring spawn areas because hydrocarbon concentrations were elevated in water but not in intertidal sediment. Mackay et al. (1980) suggested the major toxic effects of an oil spill may be from hydrocarbons dissolved from dispersed oil. Oil can be dispersed in the water column by wind or wave activity (Mackay et al. 1980; Payne et al. 1991; Wolfe et al. 1994), and three days after the *Exxon Valdez* spill a three-day storm dispersed substantial quantities of oil into the water (Wolfe et al. 1994). This dispersed oil was clearly accumulated by mussels tethered at depths to 25 m (Short and Harris 1996*b*). Phytane was present in 77% of Naked Island area mussels and 58% of Montague

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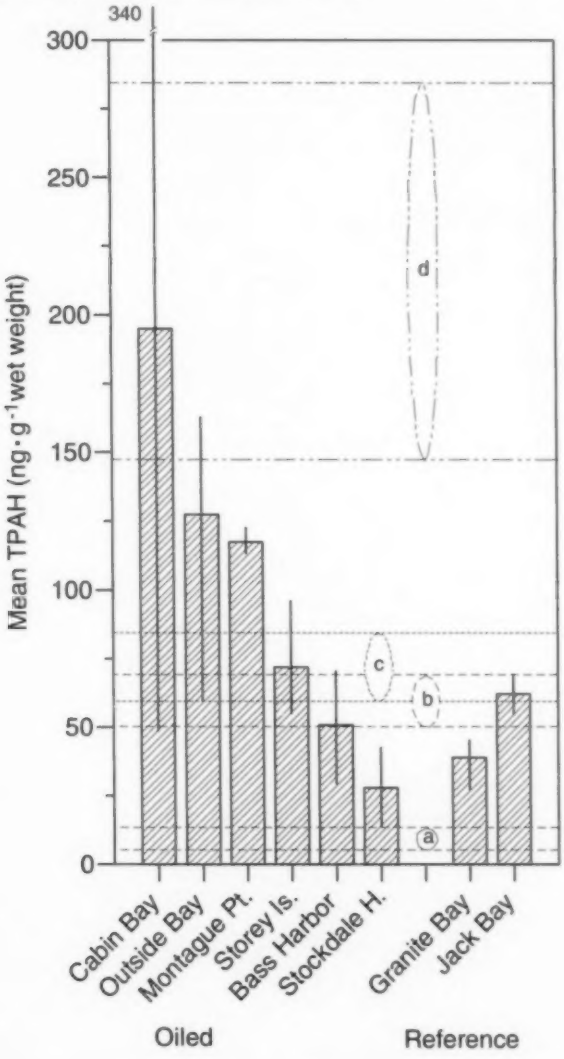
Fig. 6. Mean total polynuclear aromatic hydrocarbon (TPAH) concentration in Pacific herring eggs-on-kelp (Pearson et al. 1995*a*). Error bars indicate range of means by tide zone. Background TPAH concentrations in herring egg tissue estimated from (*a*) laboratory exposures (Carls et al. 1999) and (*b*) PWS (Pearson et al. 1995*a*) are indicated. To compensate for differences in background concentrations, estimates of damaging concentrations were scaled to field background estimates. (*c*) The minimum estimated zone of concentrations sufficient to cause abnormalities is based on several estimates of the lowest observed effective concentrations in the laboratory (Carls et al. 1999) applied to two estimates of field background concentrations (see text). (*d*) Estimation of minimum concentrations sufficient to be directly lethal were calculated similarly.



Island area mussels in 1989, confirming the presence of particulate oil in the majority of samples (Short et al. 1996). Dispersion of oil into water greatly increases the surface area of the oil, thus accelerating the weathering processes described by Short and Heintz (1997) and increasing the concentrations of dissolved hydrocarbons, particularly of the higher molecular weight, less soluble compounds.

All available evidence indicates that the primary source of herring egg contamination by oil in PWS was water mediated. The PAH in nearly all eggs-on-kelp samples from PWS (Pearson et al. 1995) must have been accumulated from surrounding water because (*i*) TPAH were present in the water column (Neff and Stubblefield 1995; Short and Harris 1996*a*); (*ii*) TPAH were accumulated from water by both caged (Short and Harris 1996*b*) and native mussels (Brown et al. 1996*a*) in spawn areas; (*iii*) composition of TPAH in the only herring egg sample available to us (Short et al. 1996) is consistent with contamination by dissolved PAH; (*iv*) <4% of herring eggs had visible tarry oil deposits (Pearson et al. 1995); and (*v*) oil in beach sediment was infrequently encountered and present only at low concentrations where detectable (Pearson et al. 1995; Short and Babcock 1996; Short et al. 1996). The argument put forward by Pearson et al. (1999) that the ascites (an accumulation of fluid in the body cavity) observed in herring larvae in PWS by Marty et al. (1997) must have resulted from direct coating of eggs by oil is unlikely. A coating incidence of <4% cannot explain the >16% elevation in the incidence of ascites in oiled areas. Furthermore, Hay et al. (1995) reported that herring eggs in direct contact with oil invariably died, an observation corroborated by Pearson et al. (1995), who found higher mortality in eggs coated with oil at Cabin Bay. Thus, the damaged larvae observed by Marty et al. (1997) likely resulted from exposure of eggs to aqueous PAH, not oil coating.

EVO concentrations in mussels and herring eggs were correlated in 1989

Total PAH concentrations in mussels collected by Brown et al. (1996*a*) from spawn sites were correlated with TPAH concentrations in herring eggs (Pearson et al. 1995) at the same sites, justifying the use of mussel data as surrogates for herring egg exposure to EVO in PWS. Brown et al. (1996*a*) and Hose et al. (1996) did not report TPAH concentrations in herring eggs, but rather used TPAH concentrations in mussels adjacent to herring egg collections as surrogate measures of hydrocarbon exposure of herring eggs because mussels and other suspension-feeding bivalves are frequently used for monitoring sporadically distributed hydrocarbons in seawater (e.g., NRC 1980; Wolfe et al. 1981; Armstrong et al. 1995). To determine if using TPAH in these mussels as surrogates for herring egg exposure was justifiable, mussel and herring egg data from sites sampled in common by both research teams were regressed (Fig. 7). These sites included Cabin Bay, Outside Bay, Storey Island, Bass Harbor, and Stockdale Harbor. (Total PAH data for mussels in Stockdale Harbor were collected by the U.S. Fish and Wildlife Service and reported by Short et al. (1996). Transects O1, O14, and O15 (McGurk et al. 1990; Brown et al. 1996*a*) were considered outside areas of overlap and were not included (Fig. 2)). Mean TPAH concentrations in mussels (Brown et al. 1996*a*) were correlated with those in herring eggs (Pearson et al. 1995) ($r^2 = 0.92$, $P = 0.011$) (Fig. 7). Bienert and Pearson (1995) previously argued that mussel data may be of limited value in estimating exposure

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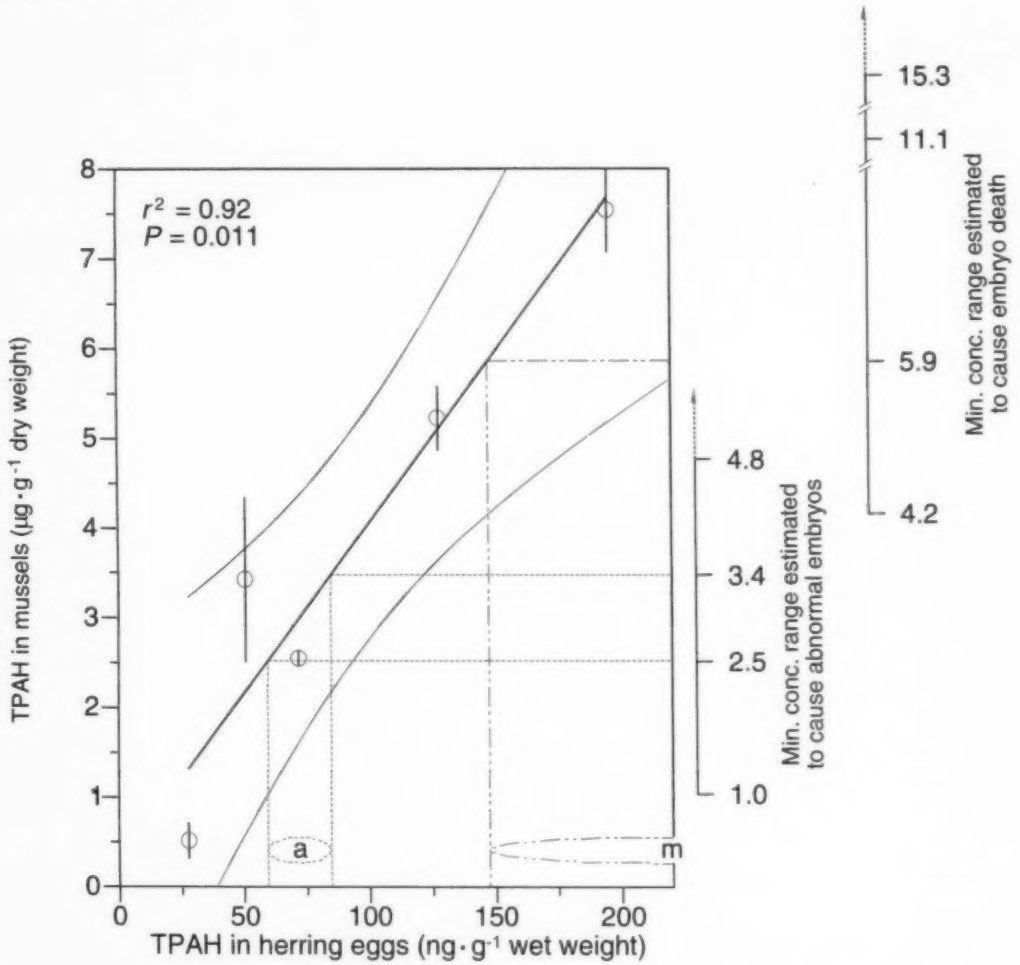
Fig. 7. Mean total polynuclear aromatic hydrocarbon (TPAH) concentrations in herring eggs-on-kelp (ng·g^{-1} wet weight; Pearson et al. 1995) and in adjacent mussels (μg·g^{-1} dry weight; Brown et al. 1996*a*) were correlated (r^2 = 0.92; bounding curves are the 95% confidence bands). Data are means ± standard error. Minimum concentrations in mussels sufficient to predict damage in herring embryos were estimated from minimum concentrations sufficient to cause (*a*) abnormalities or (*m*) mortality in Prince William Sound herring embryos (see Fig. 6 and text).



of herring eggs deposited subtidally, but demonstration (*i*) that hydrocarbons were available to subsurface marine fauna after the *Exxon Valdez* spill (Short and Harris 1996*b*); (*ii*) that TPAH concentrations in herring eggs were correlated across tide zones; and (*iii*) that TPAH concentrations in mussels were correlated with those in herring eggs suggests otherwise. Thus, the use of TPAH concentrations in mussels as surrogates for herring egg exposure to EVO in PWS by Brown et al. (1996*a*) and Hose et al. (1996) was valid.

Oil effects in PWS herring eggs

Adverse reactions of Pacific herring eggs to EVO in PWS were documented at hatching and in newly hatched larvae by NRDA studies (Brown et al. 1996*a*), but the industry study concluded that oil effects were generally negligible (Pearson et al. 1995). Response data in all studies focused primarily on the condition of larvae hatched from exposed eggs. (Eggs naturally spawned in PWS were collected late in development by NRDA and industry researchers and incubated in laboratories until hatch.) Premature hatch (Brown et al. 1996*a*) and abnormalities in Pacific herring larvae (Hose et al. 1996), effects consistent with exposure to crude oil (e.g., Smith and Cameron 1979; Weis and Weis 1989; Carls et al. 1999), were significantly more frequent in oiled areas than in reference areas of PWS. Compared with reference embryos, oiled embryos hatched earlier, producing less mature larvae (Brown et al. 1996*a*). The proportion of larvae with jaw deformities varied significantly with oil concentrations in resident mussels (Brown et al. 1996*a*; Hose et al. 1996). Oiled larvae were longer at hatch, but weighed less than reference larvae (Brown et al. 1996*a*). Hose et al. (1996) found that the severity of skeletal, craniofacial, and

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finfold abnormalities was significantly higher in oiled areas compared with a reference site, Fairmont Bay. The severity of skeletal abnormalities, certain types of craniofacial defects (jaw abnormalities, microphthalmia, and absence of otic capsules), and the total severity index were significantly correlated to log-transformed TPAH concentrations in adjacent mussels (Hose et al. 1996). Not clear is why Pearson et al. (1995) generally did not observe similar effects in embryolarval herring; possibly the statistical power of these tests was too low. The only correlation to oil that Pearson et al. (1995) found was a reduction in the proportion of developed eggs at Cabin Bay. Comparison of biological responses among sites by Pearson et al. (1995) was limited to a single statistical contrast between oiled and reference areas, but because mean TPAH concentrations in eggs-on-kelp (and in mussels) varied considerably among oiled sites, this comparison may have missed significant intersite variation. Because Pearson et al. (1995) only presented the proportions of biological response variables without reporting the total number of individuals examined, and generally did not provide site-specific and tide zone detail, direct comparison with NRDA data is not possible.

Oil effects in laboratory-exposed eggs

Extensive research has consistently demonstrated that aqueous exposure of fish eggs to oil is damaging (e.g., see reviews by Moore and Dwyer 1974; Neff and Anderson 1981; Weis and Weis 1989); however, much of this literature focused on acute toxicity and one- to two-ring aromatics. Because one-ring compounds were quickly lost from the water of PWS (Wolfe et al. 1994; Neff and Stubblefield 1995), subsequent toxicity research was specifically tailored to study constituents remaining after the initial weathering (e.g., Marty et al. 1997; Carls et al. 1999; Heintz et al. 1999).

The quantitative laboratory studies of Carls et al. (1999) provide a link between field and laboratory toxicity for embryolarval herring and can be used to hindcast biological responses in PWS to EVO in 1989. Herring eggs were exposed to concentration series of less weathered and more weathered oil in two consecutive experiments where seawater (32 ppt) was contaminated by passage through oiled gravel. Originally reported weathering estimates ranged from 0.04 to 1.28; amended ranges are $0.1 \leq w \leq 1.0$ (less weathered test) and $0.5 \leq w \leq 1.5$ (more weathered test). The resultant oil concentrations and composition closely modeled those observed in PWS after the spill (Short and Heintz 1997) and TPAH concentrations declined exponentially during tests. Total PAH accounted for 36–79% of total hydrocarbons dissolved in seawater in the less weathered test and 3–13% in the more weathered test. Exposure of eggs to 0.7 $\mu g \cdot L^{-1}$ TPAH of the more weathered oil caused malformations, genetic damage, mortality, decreased size, and inhibited swimming in herring larvae. Total aqueous PAH concentrations as low as 0.4 $\mu g \cdot L^{-1}$ caused sublethal responses such as edema (ascites) and immaturity consistent with premature hatching. Responses to less weathered oil, which had relatively lower proportions of high molecular weight PAH, generally paralleled those of the more weathered oil, but lowest observed adverse effect concentrations (LOAECs) were higher (9.1 $\mu g \cdot L^{-1}$), demonstrating the importance of composition. Based on total measured aqueous hydrocarbons (TPAH + total alkanes + UCM), LOAECs in the less and more weathered herring egg tests were 25 $\mu g \cdot L^{-1}$ and 14 $\mu g \cdot L^{-1}$ (previously unreported by Carls et al. 1999). Correlations between biological responses and TPAH were consistently greater than correlations to either total alkanes or the UCM.

To properly match laboratory and field results, the state of oil weathering, summarized by w, was considered. Mean w, as estimated from mussel tissue, was 3.7 (range 0.7–5.7, n = 51) during the critical egg development period in PWS (1989) and 2.3 in herring eggs (n = 1), thus generally exceeding weathering in both experiments reported by Carls et al. (1999). However, Heintz et al. (1999) provide evidence that ANSCO remains toxic with further weathering (w = 4.9), and continues to be more toxic per unit mass than unweathered oil. Thus, use of the more weathered test by Carls et al. (1999) for interpretation of field results is more justifiable than use of the less weathered test, but may underestimate the true toxicity owing to continued weathering. True toxicity may also be underestimated because exposure to ultraviolet light was negligible during laboratory incubation, but developing embryos in PWS were likely exposed to ultraviolet light from sunlight (Barron and Ka'aihue 2001), and absorption of ultraviolet light can increase PAH toxicity by two to 1000 times (Pelletier et al. 1997). Tidally influenced fluctuations in salinity were not modeled by the laboratory experiment, but could also have influenced toxicity in PWS. For example, Vines et al. (2000) found that the increased incidence of abnormalities of herring embryos exposed to creosote was less at 28 ppt than at lower salinities (16 ppt and 8 ppt).

Laboratory toxicity estimates suggest toxicity occurred in PWS

Three independent estimates of herring egg exposure to hydrocarbons in PWS in 1989, interpreted by laboratory exposures, provide evidence that the oil spill was damaging: TPAH concentrations in water, herring eggs, and mussels. These predictions of damage were confirmed by observation of larvae collected from oiled areas in PWS with abnormalities consistent with exposure to oil (Marty et al. 1997).

Water

Directly measured aqueous TPAH concentrations in PWS in 1989 exceeded concentrations detrimental to herring embryos and contaminated water contained high molecular weight compounds (e.g., phenanthrenes and chrysenes). Peak aqueous TPAH concentrations in Cabin Bay and Rocky Bay (1.9–2.6 $\mu g \cdot L^{-1}$; Short and Harris 1996*a*) exceeded LOAECs causing abnormalities in herring embryolarvae (0.4 $\mu g \cdot L^{-1}$) in controlled laboratory experiments (Carls et al. 1999). Concentrations of TPAH in surface water averaged over a wider area also exceeded LOAECs (Neff and Stubblefield 1995); intertidally spawned eggs were potentially exposed to surface sheens as tides fluctuated. Arguments that imply that EVO did not harm marine organisms because TPAH concentrations never exceeded water quality standards (e.g., Neff and Stubblefield 1995; Neff and Burns 1996; Pearson et al. 1999) are invalidated by postspill field and laboratory observations to the contrary (e.g., Hose et al. 1996; Marty et al. 1997; Carls et al. 1999).

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Herring eggs

Interpretation of 1989 field results requires comparison of observed TPAH concentrations in eggs-on-kelp from PWS with biologically significant concentrations observed in laboratory tests. Recently published estimates by Carls et al. (1999) indicate that the mean LOAEC causing abnormalities was 22 ± 4 $ng \cdot g^{-1}$ wet weight TPAH (mean of 4–16 days exposure data). Average area under the curve extending over all exposure days (0–16 days) might have been a more appropriate estimate of LOAEC causing abnormalities, 19.7 $ng \cdot g^{-1}$, and provides a lower estimate for minimum damaging exposure concentrations. The peak LOAEC causing abnormalities was 26.0 $ng \cdot g^{-1}$, providing an upper estimate of minimum damaging exposure concentrations. The published LOAEC causing embryolarval mortality was 108 ± 35 $ng \cdot g^{-1}$ in herring egg tissue (mean of 1–16 days data; Carls et al. 1999). Again, a more appropriate estimate would be the average area under the curve (0–16 days), 123 $ng \cdot g^{-1}$. The peak LOAEC causing mortality was 226 $ng \cdot g^{-1}$.

Complicating application of biologically significant laboratory concentrations to field results is a difference in field and laboratory baseline concentrations. The inferred background TPAH concentration for PWS eggs-on-kelp was the mean reference concentration (50.4 $ng \cdot g^{-1}$; our calculation). Reasons why this estimate of background concentration in eggs-on-kelp was higher in PWS (Pearson et al. 1995) than in the laboratory (10.7 ± 1.9 $ng \cdot g^{-1}$; Carls et al. 1999) are not clear, but the possibility of contamination at reference sites in PWS cannot be discounted. Other possibilities include differences in analytical and sampling procedures (e.g., kelp was included in samples analyzed by Pearson et al. (1995), but not in laboratory samples (Carls et al. 1999)). To compensate for these background differences, we calculated the difference between each laboratory estimate and the mean laboratory background concentration, and added these differences to the field background concentration. To estimate an upper limit for minimum concentrations causing abnormalities, we increased the estimated background concentration for PWS to the maximum mean concentration reported for any reference tide zone in 1989 (69 $ng \cdot g^{-1}$; Pearson et al. 1995).

Direct measurement of TPAH in Pacific herring eggs in 1989 (Pearson et al. 1995), interpreted by recent experiments (Carls et al. 1999), suggests that exposure of eggs to EVO in five of six oiled sites in PWS was detrimental. In contrast, Pearson et al. (1995) concluded that mean TPAH concentrations in eggs-on-kelp from PWS in 1989 did not differ significantly between oiled and reference areas and that only three samples, representing two sites, were contaminated with EVO. Estimated minimum concentrations that caused abnormalities in PWS herring eggs, 59.4–84.6 $ng \cdot g^{-1}$ wet weight, included four or five of six oiled sites; observed concentrations in Bass Harbor fell within the zone of uncertainty (Fig. 6). However, larvae at Bass Harbor were shorter and grew more slowly than larvae from reference sites, and a number of histopathological and cytogenetic scores were elevated (finfold, cytologic, and craniofacial lesions, yolk, ascites, and anaphase aberration; Marty et al. 1997). McGurk and Brown (1996) found mean egg–larval mortality at Bass Harbor was greater than at reference sites, also demonstrating that these effects were biologically significant. Although growth differences could have been influenced by unrelated site-specific variables, all of these differences are consistent with exposure to oil and confirm that the model accurately predicts occurrence of negative effects, including those in Bass Harbor, a site within the zone of uncertainty. One or two sites may have experienced minimum concentrations sufficient to be directly lethal (147–285 $ng \cdot g^{-1}$), Cabin Bay and portions of Outside Bay (Fig. 6). Observation of eggs in contact with oil at these two sites (Pearson et al. 1995) together with the observation by Hay et al. (1995) that eggs in direct contact with oil invariably die early in development suggests the accuracy of our interpretation that oil concentrations at these two sites could be directly lethal. We conclude that oil-induced abnormalities, which likely presaged delayed mortality, were plausible at 83% of the oiled sites sampled by Pearson et al. (1995) and were confirmed in the oiled site least likely to produce significant differences, Bass Harbor (Marty et al. 1997).

Mussels

Interpretation of TPAH concentrations in mussels, which were correlated with TPAH concentrations in herring eggs, provides a third estimate of toxicity, and demonstrates that damage to herring embryos was plausible in Rocky Bay, a site not sampled by Pearson et al. (1995, 1999). Minimum concentrations in mussel tissue sufficient to predict adverse reaction in adjacent herring embryos were estimated from the regression between TPAH concentration in herring eggs-on-kelp and mussels (Fig. 7). Ranges of critical exposure concentrations estimated from TPAH in mussel tissue are greater than corresponding ranges estimated directly from herring eggs due to uncertainties in the linear fit. Minimum concentrations sufficient to predict abnormalities ranged from 1.0 to 4.8 $\mu g \cdot g^{-1}$ dry weight and those sufficient to be directly lethal ranged from 4.2 to 15.3 $\mu g \cdot g^{-1}$ (Fig. 7). The mean TPAH concentration in Rocky Bay mussels was >3.4 $\mu g \cdot g^{-1}$ but <4.8 $\mu g \cdot g^{-1}$, suggesting negative effects were plausible. Indeed, Rocky Bay larvae were shorter and grew more slowly than larvae from reference sites and exhibited histopathological and cytogenetic damage consistent with oil exposure (Marty et al. 1997). McGurk and Brown (1996) found mean egg–larval mortality at Rocky Bay was greater than at reference sites, demonstrating that these biological effects were consequential. Elevated TPAH concentration in Naked Island mussels was also consistent with observed larval damage.

Laboratory exposure experiments provide useful modeling of real-world effects

That the laboratory results of Carls et al. (1999) predicted response of PWS herring larvae to EVO with considerable precision also demonstrates that these tests accurately model real-world spill conditions and are not misleading, as implied by Neff et al. (2000). Hydrocarbons other than PAH may contribute to petroleum toxicity, but the toxicity of PAH is well recognized. At a minimum, TPAH serves as an index of the presence of oil and was the only measure of oil concentration common to the studies presented in this synthesis.

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In most cases, acute toxicity of a petroleum product is directly correlated to its content of soluble aromatic derivatives (Anderson et al. 1974; Moore and Dwyer 1974; Neff 1979). Neff et al. (2000) reported that PAH accounted for 3–94% of estimated hazard indices of four oils and that the contribution increased with weathering: TPAH contribution to toxicity in the most weathered fractions ranged from 57 to 94% (omitting a 99% observation for one oil because of analytical errors). Phenols contributed <15% to the toxicity in any test by Neff et al. (2000), and did not vary consistently as a function of weathering. Similarly, Barron et al. (1999) also found that the least toxic of three oils had by far the highest phenol concentration. Retention of the higher molecular weight, more refractory, and more toxic PAH in aqueous solution as the oil weathers appears to best explain the increased toxicity of weathered oil (Carls et al. 1999; Heintz et al. 1999, 2000).

Although microorganisms throughout the marine environment have evolved the ability to metabolize hydrocarbons (Atlas 1981; Floodgate 1984), and the resultant metabolic byproducts can be toxic (Middaugh et al. 1998; Shelton et al. 1999), the estimated microbial contribution to toxicity in tests by Carls et al. (1999) is minimal. Concentration of the water-soluble fraction (WSF) varied among microbial cultures and increased with time (Middaugh et al. 1996, 1998; Shelton et al. 1999); concentration changes explained >70% of the variation in toxicity (our analysis). Furthermore, microbial degradation of oil is typically nutrient limited (e.g., Gibbs and Davis 1976; Atlas 1995), as was the case in water-column tests (Middaugh et al. 1996, 1998; Shelton et al. 1999) and in experimentally oiled sandy-gravel columns (Gibbs and Davis 1976). Biodegradation rates may be increased by five to 10 times by nutrient additions (Bragg et al. 1994; Atlas 1995; Shelton et al. 1999). The time required for hydrocarbon-using microbes to begin to degrade substantive quantities of hydrocarbons in beach substrate may require roughly one to three weeks (Gibbs and Davis 1976). Degradation rates for a given microbial population can be far less at low temperatures than at high temperatures (e.g., ZoBell 1969; Gibbs and Davis 1976; Atlas 1981). Experimental conditions used by Carls et al. (1999) were less favorable for microbial growth than those by Middaugh et al. (1996, 1998) and Shelton et al. (1999): temperatures were colder, (4–7°C versus 20°C), water flow was rapid (5–6 $L \cdot min^{-1}$ versus static), and nutrients were not added. Dividing the potential contribution of metabolic byproducts (<30%; estimated from Shelton et al. 1999) by 3–5 to account for slower degradation rates without nutrient supplement (Atlas 1995) and by 3.7 to account for colder temperatures (estimated from Gibbs and Davis 1976), we estimate that metabolic byproducts accounted for <2–3% of the toxicity reported by Carls et al. (1999).

Prediction of oil effects in PWS herring eggs-on-kelp using laboratory results was restricted to 1989 because the primary oil reservoir, PWS water, was essentially uncontaminated in spawn areas after 1989 (Fig. 1), except at two islands where intertidal sediment had been heavily oiled. Total PAH concentrations in mussels at Cabin Bay and Rocky Bay fell in 1989 and remained at baseline in the following years (Figs. 1 and 4). EVO was not verifiable in 47 of 48 mussel samples collected from oiled spawn sites in 1990 (Short et al. 1996). Biologically available EVO was plausible in some (7 of 13) mussel samples at Green Island (0.02–4.8 $\mu g \cdot g^{-1}$) and Smith Island (0.07–5.3 $\mu g \cdot g^{-1}$), sites that received spawn in 1990 but not in 1989; however, samples were too weathered and the source was verifiable only once ($w = 7.70$ at Green Island, Short et al. 1996). These areas received a relatively small proportion of the total spawn (roughly 6% at Green Island, and 3% at Smith Island; our estimates from Brady et al. (1991)). Because mussels are effective water samplers, there is little reason to believe that aqueous PAH concentrations were elevated a year or more after the spill at most herring spawn sites, and Neff and Stubblefield (1995) reported little or no TPAH in PWS water in 1990 (Fig. 1). The 1990 herring eggs-on-kelp data are perplexing because there is continued evidence of elevated TPAH concentrations at both oiled and reference sites in PWS (Pearson et al. 1995), yet concentrations in eggs did not correlate with those in mussels at sites in common geographically and temporally ($r^2 = 0.19$, $P = 0.562$, $n = 4$). The composition of this contamination in PWS and at Sitka Sound reference sites is unknown to us because the data were not published (Pearson et al. 1995). Available evidence suggests <10% of the herring eggs were exposed to EVO in 1990. Thus, application of laboratory results (Carls et al. 1999) to PWS data is not advisable beyond 1989 and the lack of embryo response in 1990 (Hose et al. 1996) suggests contaminant hydrocarbon composition was not the same as in 1989.

Differences in industry and NRDA perspectives explain conflicting conclusions

Some of the differences in industry and NRDA embryo-larval studies may be explained by intensity of effort. The parallel studies in 1989 lead by Pearson et al. (1995) (industry) and McGurk (1992) (NRDA) both initially reported minor impacts. Industry studies stopped at this point, but NRDA studies were extended. Hose et al. (1996) examined larvae hatched in the McGurk study for genetic and morphological damage. Field efforts to directly examine PWS herring adults and larvae were also added (McGurk and Brown 1996; Marty et al. 1997, 1999). These extended studies demonstrated that significant damage occurred in embryolarvae from oiled areas compared with those from reference areas. In their update of McGurk (1992), McGurk and Brown (1996) reported that differences in growth and mortality rates between oiled and nonoiled areas supported the hypothesis of injury to herring embryos and larvae. The philosophy behind the science was apparently different; industry initially found minor effects, stopped testing, and concluded the spill caused little damage, but the NRDA group was not convinced that initial equivocal results could be interpreted as not harmful, continued testing, and ultimately concluded that there were major spill effects.

The effects of the EVO spill on herring eggs extended beyond the restricted areas where oil was in contact with eggs. Pearson's group concludes that oil must adhere to (or coat) herring eggs to cause effects (Pearson et al. 1985, 1995, 1999), but this perspective conflicts with the large body of literature indicating significant oil toxicity in the absence of coating (e.g., Moore and Dwyer 1974; Neff and Anderson

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1981; Grimmer 1983). Hay et al. (1995) provide a link between coating and chemical toxicity effects. They report that direct contact with oiled substrate invariably killed eggs, but because oiled substrate negatively impacted many eggs even though most were not in direct contact with oil, the primary exposure mechanism must have been via contaminated incubation water (Hay et al. 1995). Dissolved hydrocarbons are clearly toxic to fish embryos (e.g., Mironov 1967; Kuhnhold 1974; Carls et al. 1999). Our conclusion is that herring eggs in PWS were exposed primarily to dissolved oil in water and that this was the principal route of toxicity after the *Exxon Valdez* spill.

Although both industry and NRDA researchers relate egg exposure to TPAH concentrations, the two groups draw upon different literature to interpret EVO toxicity and reach opposite conclusions concerning its toxic potential in PWS. Industry researchers estimated the toxic potential of dissolved EVO using water-soluble and water-accommodated fraction research (Pearson et al. 1999; Neff et al. 2000). Aromatic compounds present in short-term, unweathered water-soluble or water-accommodated fraction tests are typically dominated by single-ring compounds (e.g., Rice et al. 1979; Neff and Anderson 1981; Rice et al. 1987). Neff et al. (2000) conclude that "under mild conditions with little physical dispersion of petroleum into the water column, volatile monocyclic aromatic hydrocarbons dissolving from the surface slick would be the main contributors to any toxicity observed in water column organisms". In their review, Pearson et al. (1999) argue that "the main hydrocarbons present in the water-soluble fraction include the low relative molecular mass PAHs that are responsible for most of the toxicity of crude oil (Rice et al. 1977; Neff and Stubblefield 1995)" and conclude that the WSF used in toxicity tests can be compared with the composition of the hydrocarbon fraction present in the PWS water column following the spill. Problems with these assertions are that (*i*) oil and water in PWS did not mix under calm conditions; (*ii*) monoaromatic hydrocarbons all evaporated rapidly from PWS; and (*iii*) toxic high molecular weight PAH were present in PWS in addition to low molecular weight PAH. Three days after the *Exxon Valdez* spill, a three-day storm dispersed substantial quantities of oil into the water and accelerated the evaporation process (Wolfe et al. 1994). By the time herring spawned, composition of oil in PWS did not resemble the WSFs because monoaromatics had evaporated (Wolfe et al. 1994; Neff and Stubblefield 1995) and high molecular weight PAH were present (Neff and Stubblefield 1995; Short and Harris 1996*a*; Short and Heintz 1997). The technique used by Carls et al. (1999), passage of water through oiled gravel, produced water with a PAH composition similar to that observed in PWS (naphthalenes through chrysenes), including the weathering patterns characterized by Short and Heintz (1997). Because high molecular weight PAH are more toxic than low molecular weight PAH (Rice et al. 1977; Neff 1979; Black et al. 1983), Carls et al. (1999) and Heintz et al. (1999) observed that PAH from EVO was about 1000 times more toxic than previously studied WSFs. Reliance of industry on traditional acute toxicity tests, which focus on narcosis induced by the most water-soluble components of oil, does not explain the suite of toxic effects in embryonic life stages caused by exposure to PAH, where the toxicity mechanism is oxidative cellular damage that can be passed on to daughter cells during development (Livingstone et al. 1990; Akcha et al. 2000). Thus, whereas industry concludes that the roughly 1 $\mu g \cdot L^{-1}$ aqueous concentrations of EVO in PWS were not toxic, NRDA researchers conclude that 1 $\mu g \cdot L^{-1}$ aqueous concentrations were sufficient to have caused significant damage.

Reassessment of the fraction of herring eggs exposed to EVO in PWS

We reassessed the fraction of PWS herring eggs exposed to EVO in 1989 by determining toxic body burdens of EVO at various oiled sites and related them to those of industry (4–10% oiled, Pearson et al. 1995, 1999) and NRDA (40–52%, Brown et al. 1996*a*, 1996*b*) (Table 1). (Zaikof Bay was included in the high estimate by Brown et al. (1996*a*, 1996*b*), but excluded from the low estimate because of insufficient chemical analysis.) Earlier we noted that the industry assessment was based on spawn distance, whereas the NRDA estimate was based on spawn biomass; the difference in estimation methods is <10%. We concur that biomass is the more appropriate measure of egg exposure and report only this measurement in the following text. Evidence from all portions of the Naked Island area suggested biologically significant oiling, i.e., TPAH concentrations in mussels and herring eggs were high enough to cause developmental abnormalities in Pacific herring embryos. Concentrations were similarly significant in the outer portion of Rocky Bay, although concentrations in the inner portion of the bay may have been below biological significance. (Total PAH concentrations in mussels in the inner portion of the bay (generally west of and including site O18 in Fig. 3) were less than the 2.5 $\mu g \cdot g^{-1}$ level estimated to signal biological significance, but TPAH concentrations in water samples collected in the eastern portion of the inner bay were consistently above 0.4 $\mu g \cdot L^{-1}$, a level that caused adverse biological response in herring embryolarvae in laboratory tests (Carls et al. 1999).) We conclude that 25–30% of the spawn biomass was oiled at biologically significant levels and that oiling was likely significant in an additional 2% of the spawn biomass at Montague Point. Oil was visually identified at Montague Point (Gundlach et al. 1990; Brady et al. 1991; Neff et al. 1995), but not documented by chemical analysis. Graveyard Point may have acted as a natural barrier to shield Stockdale Harbor from most of the oil slick, where evidence suggests that oil concentrations were too low to be detrimental to herring egg development (6% of spawn mass). There were insufficient data to determine if herring egg exposure was significant in Zaikof Bay (13% of spawn mass). Our estimate of the percentage of Pacific herring eggs exposed to biologically meaningful amounts of EVO in 1989 (25–32% of spawn mass) falls between the original estimates.

Identical oil effects were observed in older herring larvae from oiled PWS sites

Pacific herring larvae from oiled areas of PWS were also adversely affected by EVO in 1989, and the causal relationship between oil exposure and detrimental effects was confirmed through laboratory study. Major oil-associated effects in larvae captured from oiled sites in spring 1989 included small size, ascites, pericardial edema, delayed development,

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Table 1. Reassessment of percentages of Pacific herring eggs exposed to *Exxon Valdez* crude oil in Prince William Sound after the spill and comparison to previous estimates.

	Spawn		Percent	
Oiling	Distance (km)	Mass (metric tons)	Based on distance	Based on mass
Industry estimate[a]				
Moderate–heavy	0.9	NE	1	NE
Very light–light	5.2	NE	3	NE
None	152.2	NE	96	NE
Total	158.3			
NRDA estimate[b,c]				
Oiled[d]	54.7	20 886	35	40
Zaikof Bay	13.8	5 998	9	12
Not oiled	89.8	25 351	57	48
Total	158.3	52 235		
Reassessment				
Significant[e]	30.6–38.1	13 230–15 911	19–24	25–30
Likely significant[f]	2.7	984	2	2
Unknown[g]	19.0	6 823	12	13
Likely not significant[h]	8.8–16.2	3 167–5 847	6–10	6–11
None	89.8	25 351	57	48
Total	158.3	52 235		

Note: Published Natural Resource Damage Assessment (NRDA) estimations were based both on spawn distance and egg mass; the industry estimate was based only on spawn distance. NE, not estimated.

[a]Pearson et al. (1995*a*).

[b]Brown et al. (1996*a*).

[c]Brady et al. (1991).

[d]Brown et al. (1996*b*) stated range of eggs exposed to oil was 41–52%, based on spawn mass.

[e]Includes the Naked Island area plus Rocky Bay and Graveyard Point.

[f]Oiling at Montague Point was likely biologically significant and most of the spawn overlapped visibly oiled shoreline (Neff et al. 1995), but TPAH (total polynuclear aromatic hydrocarbon) concentrations were not documented by chemical analysis.

[g]Insufficient data were collected in Zaikof Bay to either support or refute exposure of Pacific herring eggs in 1989.

[h]There were indications of slight oiling in Stockdale Harbor, but the weight of evidence suggests concentrations were probably too low to damage herring eggs.

and genetic damage (Marty et al. 1997). Microscopic lesions were consistent with decreased growth and increased mortality of herring larvae collected near oiled beaches (McGurk and Brown 1996) and were similar to those observed in laboratory studies of herring larvae exposed to ANSCO as eggs (e.g., Kocan et al. 1996; Marty et al. 1997; Carls et al. 1999). Because of the low aqueous concentrations of hydrocarbons in PWS and variable environmental conditions, the link between ascites and oil exposure in field-sampled larvae was controversial (Pearson et al. 1999). Recent laboratory studies, however, provide evidence that aqueous PAH concentrations of 0.4–1 $\mu g \cdot L^{-1}$ cause ascites and edema in herring exposed as developing eggs (Carls et al. 1999), concentrations well within documented TPAH levels in PWS in April and May 1989 (Neff and Stubblefield 1995; Short and Harris 1996*a*). Growth of older larvae from offshore areas also decreased throughout PWS in 1989, but a direct link to oil exposure was not possible because these larvae likely originated from a mixture of oiled and unoiled areas (Norcross et al. 1996). In contrast, the frequency of genetic defects was low and jaw size was within normal limits in PWS larvae six years after the spill (Norcross et al. 1996). Pacific herring larvae that hatched in PWS were not studied by industry. We conclude that NRDA information on embryolarval effects, coupled with identical responses observed in the laboratory and in free-swimming herring larvae, provides consistent documentation of oil toxicity in early life stages of PWS herring in 1989. (Effects of EVO on juvenile herring were not evaluated by either group.)

Adult PWS herring accumulated hydrocarbons and exhibited histopathological changes

In 1989, adult Pacific herring in oiled areas of PWS exhibited significant oil-associated lesions and evidence of hydrocarbon exposure, but fish from reference sites did not (Moles et al. 1993; Marty et al. 1999). When the oil spill occurred, herring were beginning to congregate in shallow bays for their annual mass spawning in April. Fish from oiled sites had hepatic necrosis and elevated PAH concentrations (primarily naphthalenes) in their tissues (Marty et al. 1999). Naphthalenes were also preferentially accumulated in muscle tissue in laboratory exposures of adult herring to PAH in water (Carls et al. 2000), evidence of metabolism (Thomas et al. 1997) and differential uptake. Herring from oil-exposed areas had fewer nematode parasites in their body cavities than did fish from reference sites (apparently because stressed nematodes migrated into muscle tissue) and the potential link to acute oil exposure was confirmed through laboratory study (Moles et al. 1993). In a recent study, wild-caught Pacific herring exposed to crude oil had higher prevalence of hepatic necrosis, increased mortality, and viral hemorrhagic septicemia virus (VHSV) was isolated from exposed fish but not control fish (Carls et al. 1998). Also, aqueous PAH concentrations in the range of those

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measured in PWS in spring 1989, ≥0.6 $\mu g \cdot L^{-1}$, cause immunotoxicity in rainbow trout (Karrow et al. 1999). Although significant mortality of adult herring in 1989 as a result of the spill was never documented in PWS, lesions in fish sampled from oiled sites in 1989 were consistent with lesions in fish from which VHSV was isolated in more recent studies (Marty et al. 1998, 1999), and a small fraction of the population probably died as a result of this viral outbreak. In 1990 and 1991, fish sampled from oiled sites had neither oil-related lesions nor significant PAH concentrations in their tissue (Marty et al. 1999).

Long-term spill consequences were indirect or not associated with the spill

Adult biomass in PWS was at historically high levels in 1989 and continued at high levels through 1992. During this period, the spawning population was composed mostly of fish that were adults during the 1989 oil spill because PWS herring first spawn when they are 3 years old.

Contrary to predictions made before the spring of 1993, the adult Pacific herring population of PWS declined more than 75% from an estimated 11×10^7 kg in 1992 to 1.7×10^7 kg in 1994, and most of the decline occurred during the winter of 1992–1993 (Marty et al. 1998). High prevalence of ulcers in surviving fish in spring 1993 provided strong evidence that the population decline was associated with a disease outbreak. Although natural variation in clupeid population size can be large and unpredictable (Blaxter and Hunter 1982; Cole and McGlade 1998), recruitment failure did not explain the collapse (fish from all year classes were involved), and the magnitude of the 1992–1993 collapse suggests external factors played a critical role.

Several hypotheses have been advanced to explain the population collapse including both spill-related and natural reasons, and combinations of the two. These hypotheses are discussed in detail by Pearson et al. (1999) and Carls et al. (2001*a*) and have not been repeated here. Our perspective is that important stressors responsible for the collapse of the adult herring population in 1993 included large population size, poor overwinter fish condition, and possibly environmental conditions, but links between the EVO spill and delayed population response are tenuous. The herring population in PWS may have approached or exceeded carrying capacity between 1988 and 1992, thus the risk of a disease epizootic was high. Although the major agent in the epizootic, VHSV, can be induced by acute exposure to oil and can cause rapid mortality in infected adult herring, delayed reaction of herring to oil toxicity in 1993 is unlikely. The estimated additional increase in population in 1989 resulting from the postspill fishery closure was small, but the precipitous 1993 collapse of the population near its carrying capacity underscores the importance of formulating resource management decisions that integrate knowledge of the historical fishery. Populations at carrying capacity that are exposed to additional stress, such as oil, may be at greater risk than populations below carrying capacity, in addition to indirect effects such as postspill management decisions. Environmental conditions unrelated to the oil spill, such as prewinter prey availability and winter starvation, may have also been important contributing factors. Probably no single factor can completely explain the population collapse.

Summary and conclusions

Reassessment of response of Pacific herring to oil in PWS suggests that observed impacts could not have been predicted from routine toxicological monitoring methods in use at the time of the spill. This review emphasizes the need to thoroughly study pollutant exposure, adequately characterize pollutant composition, estimate toxic effects using appropriate laboratory models, critically examine sensitive life stages over appropriate time intervals, and place the findings within broad ecological perspectives. That the PWS oil spill occurred in a nearly pristine environment (Karinen et al. 1993) yields much information that can be advantageously used in situations complicated by previous habitat degradation. Adverse biological responses were observed orders of magnitude below expected responses in part because the spill chemistry had not been accurately predicted, in part because sensitive early life stages were exposed during critical development periods, and because sufficient funding and controversy ensured intensive study. Because oil typically persists for at least several days after a spill, the research paradigm should shift away from an emphasis on mechanisms of acute toxicity (such as narcosis) to long-term toxicity (e.g., oxidative cellular damage). As a result of this reassessment, we recommend that safety standards for dissolved PAH should be revised to reflect a new toxicity threshold of <1 $\mu g \cdot L^{-1}$ (part per billion) TPAH to adequately protect aquatic organisms and habitat. Assessment of risk should also consider population dynamics (e.g., a population at carrying capacity may be at greater risk than one below capacity), season (e.g., spill effects are more deleterious when they impact critical early life stages), location of the spill (such as spawning grounds), local ecology, hydrographic conditions, and large-scale ecological processes. Results of the many *Exxon Valdez* studies have broad applicability to other situations, such as the combined industrial and non-point-source runoff that results in elevated aquatic PAH loads near urban areas (Rice et al. 2001).

We do not recommend that routine monitoring of hydrocarbons in other species (such as mussels) be substituted for direct measurements in the species of interest (e.g., herring eggs). For this particular spill we made use of the data available, and the close correspondence between TPAH concentrations in herring eggs and mussels allowed us to verify the assertions of NRDA researchers that mussels were adequate proxies for direct measurement in herring eggs. However, we have used the mussel data with caution and based our conclusions regarding adverse reaction of PWS herring embryos to oil principally on observed TPAH concentrations in those eggs, responses to similarly low TPAH concentrations in experimentally exposed herring eggs, and directly observed effects in PWS larvae. That TPAH concentrations in herring eggs and mussels were correlated suggests that correlations may be found in other spills, but the nature of such correlations may depend on many factors, such as mixing energy and duration, temperature, salinity, oil viscosity, and organic content of receiving water (Rice et al. 1977; Gustafson and Dickhut 1997), and thus may not be consistent among oil spills. Mussels, which are filter feeders, preferentially accumulate particulate or colloidal oil (Short and Harris 1996*b*; Axelman et al. 1999), but the majority of exposed herring eggs in PWS likely responded to dissolved oil. Particulate

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and dissolved hydrocarbon concentrations can be related in that the large surface area presented by small particles will accelerate dissolution (Short and Heintz 1997). However, because petroleum hydrocarbons are sparingly soluble, contact time is also very important, and when contact time is short, the correlation between particulate and dissolved compounds may be substantially different than when longer contact times are involved (Axelman et al. 1999). In general, routinely monitored "indicator" species (e.g., mussels) can identify likely environmental problem areas, but should not simply be substituted for direct measurements in the species of interest.

Differing perspectives between industry and NRDA researchers led to disparate conclusions concerning the consequences of the *Exxon Valdez* spill to PWS herring in 1989. Because initial results were equivocal and environmental oil concentrations (roughly 1 $\mu g \cdot L^{-1}$ aqueous TPAH) were below previously published toxicity thresholds, industry researchers stopped testing and concluded that the spill caused little damage. In contrast, NRDA researchers were unconvinced that initial results could be interpreted as harmless and continued their study, experimentally determining that 1 $\mu g \cdot L^{-1}$ aqueous TPAH concentrations can cause embryo abnormalities and mortality and ultimately concluded that short-term spill effects were major.

This reassessment shows that the short-term consequences of the spill were detrimental to herring in PWS, but that possible long-term consequences of the spill are difficult to discern. Results from these analyses have refined the nominal classification of visually oiled spawn areas using site-specific chemical evidence. Because TPAH concentrations in mussels were correlated with TPAH in herring eggs (justifying their use as a surrogate index of exposure by NRDA researchers), we inferred that herring eggs from almost all spawning sites within the oil trajectory in 1989 were exposed to EVO in the water column. The incubation of herring eggs approximately coincided with peak aqueous hydrocarbon concentrations in PWS and the bioaccumulated oil was detrimental to 25–32% of the spawned biomass. Herring larvae collected from oiled PWS areas throughout spring 1989 were malformed, small in size, and had higher mortality rates than in reference areas, consistent with field measurements of EVO above laboratory toxicity thresholds. Embryolarval toxicity appeared to be limited to 1989 and the adult herring biomass continued at high levels through 1992. The 1993 population collapse was likely caused by a combination of factors including high population size, disease, and suboptimal nutrition, but indirect links to the spill cannot be ruled out.

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PERSPECTIVE

Ecological hypotheses for a historical reconstruction of upper trophic level biomass in the Baltic Sea and Skagerrak

Brian R. MacKenzie, Jürgen Alheit, Daniel J. Conley, Poul Holm, and Carl Christian Kinze

Abstract: Fish and marine mammal populations in the Baltic Sea and Skagerrak have undergone major fluctuations over the past five centuries. We summarize how these fluctuations may have depended on various forms of predation (e.g., cannibalism, fishing, hunting) and environmental processes. The best-documented long-term fisheries in this region are the herring (*Clupea harengus*) fisheries near Bohuslän, western Sweden, and in the Øresund. These fisheries have been important since at least the 1200s and appear to be partly climatically driven. However, in the rest of the Baltic, information about fisheries for herring and other fish species is rare until after 1900. During the 20th century, while the Baltic underwent eutrophication, the biomass and landings of three fish species (cod (*Gadus morhua*), herring, and sprat (*Sprattus sprattus*)) all increased, whereas the biomass of marine mammals (grey seals (*Halichoerus grypus*), ringed seals (*Phoca hispida*), harbour porpoises (*Phocoena phocoena*)) decreased. The relative roles of exploitation, marine mammal predation, and environmental variability (e.g., eutrophication, major inflows of saline water, climate change) on the long-term dynamics of key fish species is not clear and requires increased collaboration among historians, fisheries and marine mammal ecologists, oceanographers, and climatologists.

Résumé : Les populations de poissons et de mammifères marins de la mer Baltique et de Skagerrak ont subi d'importantes fluctuations au cours des cinq derniers siècles. On trouvera ici une synthèse qui montre comment ces fluctuations ont pu être reliées à diverses formes de prédation (e.g., cannibalisme, pêche, chasse) et aux processus environnementaux. Les pêches commerciales les mieux connues sur une longue période sont celles de hareng (*Clupea harengus*) près de Bohuslän dans l'ouest de la Suède et d'Øresund. Ces pêches sont importantes depuis au moins le 13^{e} siècle et elles semblent être au moins en partie sous contrôle climatique. Cependant, les données sur la pêche au hareng et aux autres poissons dans le reste de la Baltique sont rares avant 1900. Au cours du 20^{e} siècle, alors que la Baltique s'eutrophisait, la biomasse et les débarquements de trois espèces de poissons (la morue (*Gadus morhua*), le hareng et le sprat (*Sprattus sprattus*)) ont augmenté et la biomasse des mammifères marins (phoques (*Halichoerus grypus*, *Phoca hispida*), marsouins communs (*Phocoena phocoena*)) a diminué. Les rôles relatifs de l'exploitation, de la prédation par les mammifères marins et la variabilité de l'environnement (e.g., l'eutrophisation, les apports majeurs d'eau salée, les changements climatiques) sur la dynamique à long terme des principales espèces de poisson est peu évidente; pour résoudre le problème, il faudrait une collaboration accrue entre les historiens, les écologistes des pêches, les spécialistes des mammifères marins, les océanographes et les climatologues.

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B.R. MacKenzie.[1] Department of Marine Ecology and Aquaculture, Danish Institute for Fisheries Research, Kavalergården 6, DK-2920 Charlottenlund, Denmark.
J. Alheit. Baltic Sea Research Institute, Seestrasse 15, D-18119 Rostock, Germany.
D.J. Conley. Danish National Environmental Research Institute, DK-4000 Roskilde, Denmark.
P. Holm. Centre for Maritime and Regional History, The University of Southern Denmark, Niels Bohrs Vej 9-10, DK-6710 Esbjerg, Denmark.
C.C. Kinze. Fisheries and Maritime Museum, Tarphagevej 2, DK-6710 Esbjerg V, Denmark, and Zoological Museum, University of Copenhagen, Copenhagen, Denmark.

[1]Corresponding author (e-mail: brm@dfu.min.dk).

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Introduction

Understanding the causes of variations in abundance of upper trophic level animals is key to their long-term sustainability and that of the ecosystems in which they live (Steele and Schumacher 2000). However, in many cases, it is difficult to determine why abundances vary. Are the variations mainly due to anthropogenic factors (e.g., fishing mortality and hunting), or do climatic and other environmental factors (e.g., unusual sea temperatures) have a role? Attempts to distinguish these two major causes of variations in animal abundance, and how they interact, are limited by the relatively short time series (typically 20–30 years) of reliable fishery and environmental datasets available. As a result, statistical tests have little ability to resolve differences, and inferences based on such tests may not be applicable to other time periods because of long-term environmental variations (Frank 1991).

This dilemma can often be circumvented by considering how fish and marine mammal populations have varied during historical periods when exploitation rates were lower and (or) abundances were higher than at present (Francis and Hare 1994; Hutchings and Myers 1995). Methods for investigating variations in abundance during the pre-industrial period (ca. pre-World War II) include (*i*) reconstructing catch and effort data from historical sources (e.g., records of taxes and tithes, sales of fish, lists of food provisions, reports of privileged fisheries such as shellfish; Kinze 1995; Hutchings and Myers 1995; Holm 1996) and (*ii*) examining historical sediment records in anoxic regions of the sea for the presence of fish scales of different species (Francis and Hare 1994; IGBP 1999). Both of these methods have proven to be effective in constructing reliable long-term (multidecadal) time series of fish and marine mammal abundances, and both methods have demonstrated that fish populations do undergo moderate to large fluctuations in abundance. Moreover, similar methods can also be used to develop long-term time series of important climatic variables (Euroglobec 1998; IGBP 1999).

In the Baltic Sea and Skagerrak region (Fig. 1), such methods have been applied to some fisheries (herring (*Clupea harengus*) near Bohuslän and in the Øresund) but not to others (e.g., cod in Subdivisions 25–32 of the International Council for the Exploration of the Sea (ICES)). In this report, we summarize the present knowledge of historical patterns in abundance of fish and marine mammals. Our objectives are to identify major gaps in understanding why some of these populations have fluctuated over long time scales (decades–centuries) and to demonstrate the potential that combined historical fisheries–oceanographic–socioeconomic research could have in resolving some of the outstanding issues. A new multidisciplinary approach could help disentangle how man and climate alter the structure and functioning of the Baltic Sea ecosystem and could help develop long-term fisheries and ecosystem management objectives.

The report is organized into subsections. After many of the subsections, we list some specific questions about the abundance of fish and marine mammals and their exploitation. These might provide useful entry points for reconstructing the structure and function of the upper trophic levels of the Baltic ecosystem in previous centuries and for understanding how environmental factors and exploitation might affect their abundances in future.

The Baltic Sea

General

The Baltic Sea is a semi-enclosed body of brackish water (Fig. 1) characterized by horizontal and vertical salinity gradients. The water exchange between the Baltic Sea and the North Sea involves many processes and is complicated. Stratification in the Baltic Sea is controlled by occasional salt water intrusions and river runoff (Schinke and Matthäus 1998), both of which are climatically controlled (e.g., North Atlantic Oscillation, Hänninen et al. 2000). In the interval between major inflow events, anoxic conditions can develop in the deep basins because of degradation of organic material. The aperiodic intrusions of the saline, well-oxygenated water from the North Sea and the Skagerrak can drastically change the hydrography of the Baltic Sea and even allow benthic settlement in the usually anoxic basins for short periods. Species diversity in the Baltic Sea is relatively low (Voipio 1981): many species are under permanent physiological stress because of the strong salinity and oxygen gradients in the geologically young Baltic.

Climatic influences

The Baltic is influenced by large-scale climatic variations in northern Europe. These are frequently associated with the North Atlantic Oscillation (NAO; Euroglobec 1998; Dickson et al. 2000), which is a climate index derived from the difference in air pressure between Iceland and southwestern Europe (e.g., Azores, Lisbon, Gibraltar). A high NAO index corresponds to strong meridional pressure gradients over the North Atlantic and intensified westerly winds. This situation is accompanied by an inflow of cold air masses from Alaska and Greenland that absorb heat while passing over the northwest (NW) Atlantic. As a result, the westerly winds carry air masses of moderate temperature towards Europe. A negative NAO index gives opposite conditions (i.e., reduced westerly winds and reduced inflow of cold air masses from Alaska and Greenland), allowing cold Siberian air masses to flow into eastern Europe in the absence of the inflowing air masses from Greenland. Therefore, the two extremes of the NAO can lead to mild (NAO positive) or severe (NAO negative) winters in Europe. More complete descriptions of the NAO and its effect on climate and hydrography in the North Atlantic can be found elsewhere (Dickson et al. 2000).

In the Baltic, the NAO has been shown to be correlated with variations in freshwater runoff and hence salinity (Hänninen et al. 2000). Ice conditions (Koslowski and Loewe 1994; B.R. MacKenzie and F.W. Köster, unpublished data) and water temperatures (B.R. MacKenzie and F.W. Köster, unpublished data) in the Baltic are also correlated with variations in the NAO. Climate variability influences dynamics of other biological components of the Baltic ecosystem, such as phytoplankton blooms (Helcom 1996) and mesozooplankton (Möllmann et al. 2000; Kornilovs et al. 2001) and macrozoobenthos abundance (Laine et al. 1997; Hagberg and Tunberg 2000). The impacts of the NAO on

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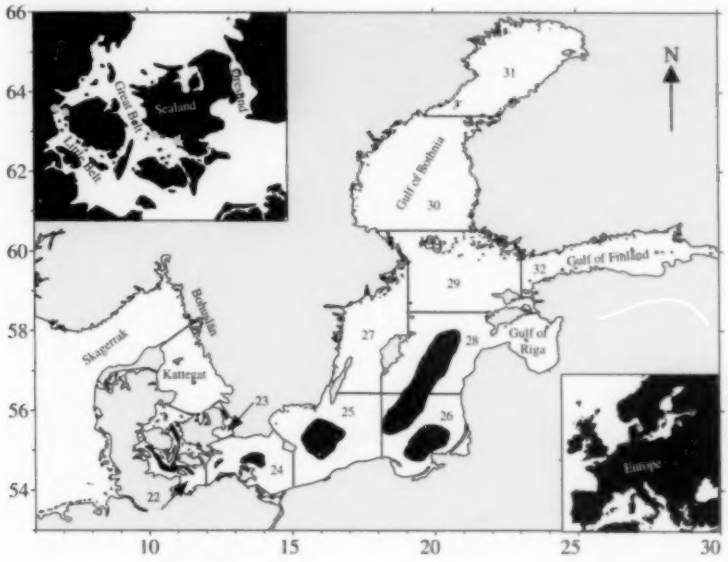
Fig. 1. Map of the Skagerrak, Kattegat, and western (ICES Subdivisions 22–24) and eastern (ICES Subdivisions 25–32) Baltic Sea. The Baltic Sea is officially defined to include the Kattegat and ICES Subdivisions 22–32 (Helcom 1996). The Baltic Proper is considered to include ICES Subdivisions 25–29. Three main cod spawning areas are shown in black in the eastern Baltic (Bornholm Basin, Gdansk Basin, and Gotland Basin located in Subdivisions 25, 26, and 26–28, respectively) as are smaller cod spawning areas in the western Baltic (see Bagge et al. 1994). The island at the border between Subdivisions 24 and 25 is Bornholm (Denmark). Inset maps show the Belt Sea – Øresund region (ICES Subdivisions 22–24) and the location of the Baltic Sea relative to the European continent and adjacent seas. Shaded dots (N = 7) on the Belt Sea – Øresund map indicate major historical catch sites for harbour porpoise (*Phocoena phocoena*; Kinze 1995).



fish populations in the Baltic Sea and the Skagerrak will be described below in further detail.

Eutrophication

Eutrophication was one of the major environmental changes in the Baltic during the 20th century (Elmgren 1989). Increased nutrient loading (e.g., resulting from agricultural use of fertilizers) has reduced water column visibility and the depth limit of macroalgae (Kautsky et al. 1986) and has increased the occurrence and duration of hypoxia or anoxia (Elmgren 1989).

Eutrophication has also directly affected Baltic fish ecology, although true cause–effect relationships are difficult to resolve (Hansson 1985; Parmanne et al. 1994; Helcom 1996). This difficulty is due partly to the relatively short time series of eutrophication-related variables (e.g., chlorophyll, nutrients) and fish biomasses and the complicated effects that eutrophication has on ecosystem structure and functioning (Hansson 1985; Helcom 1996). However, Thurow (1997) showed that herring and sprat (*Sprattus sprattus*) biomass in the Baltic Proper increased in the 1900s at a time corresponding approximately to the intensification of eutrophication. In the Kattegat, the annual yield of the fishery has increased since the 1950s along with primary production rates consistent with primary production – fishery yield relationships determined for other areas (Nielsen and Richardson 1996).

Eutrophication affects the diets of both demersal and pelagic fish species in the Baltic. For example, diets of demersal fish species in the eastern Baltic (ICES Subdivisions 25–32) and the Kattegat change because hypoxia alters the infaunal species that are prey for demersal fish (Bagge et al. 1994; Pihl 1994) or cause fish to feed on pelagic prey or in shallower water (Bagge et al. 1994).

Fish and fisheries in the Øresund and the Skagerrak

In European waters, some of the best-documented long-term fisheries are those in the Øresund and Bohuslän regions in the western Baltic (ICES Subdivisions 22–24) and in the Skagerrak (Fig. 1). Both fisheries targeted herring, but the dynamics of the fisheries differ.

Medieval herring fishery in the Øresund (ICES Subdivision 23)

The herring in the Øresund and western Baltic (Subdivisions 22–24) constituted the largest medieval fishery of Europe. The Hanseatic League was instrumental in the development of commercial fisheries (12th–16th centuries), and fish trading and its herring fishery was the first to reach the level of a real industry (Sahrhage and Lundbeck 1992; Alheit and Hagen 2001). Most of this herring came from the western Baltic (ICES Subdivisions 22–24), in particular from the narrow strait between Denmark and southern Sweden (Øresund; ICES Subdivision 23; see inset of Fig. 1), where tens of thousands of tonnes of herring were extracted. Herring in Subdivisions 22–24 is comprised of two subspecies

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(*Clupea harengus harengus* L. and *Clupea harengus membras* L.), but the relative contributions of these subspecies to the medieval herring landings in the Øresund are not known.

The fishery developed before the rise of the Hanseatic League in the 13th century and constituted the League's foremost trading commodity. Interestingly, the demise of both the herring fishery and the League happened concurrently in the 16th century. The annual fishing season lasted from August to late October, and the herring were exported throughout Europe and as far as Russia and Italy (Jahnke 2000). There are estimates of annual catches up to 40 000 – 50 000 tonnes (t) in the 15th century, which easily qualified the Øresund fishery as the largest fishery in Europe in medieval times and was only surpassed by the Dutch North Sea fishery in the 16th century (Sahrhage and Lundbeck 1992; Holm and Bager 2002). The Øresund fishery reached a peak around 1525 and was reported to be declining in the 1540s. A slump in the late 16th century was followed by a short-lived recovery in the 1620s, after which time the fishery seems to have declined to a local consumption fishery. The reasons for the decline have not been investigated. By the early 20th century, Danish catches from the Øresund were between 100 and 10 000 t. Swedish catches are likely to have been of similar or larger magnitude. Any comparison must account for improvements in the drift-net technology, although improvements in gear seem to have been limited between the 16th and the 19th centuries. Given the high price of fish in medieval times, catch effort is likely to have been higher in the 15th century than in the early 20th century (Holm 1996).

Questions related to the medieval herring fishery in the Øresund

Can herring catches of the medieval period be further quantified? Are there indications for a climatic background of this fishery?

Bohuslän herring fishery in the Skagerrak

The Bohuslän herring fishery had different dynamics than those of the Øresund herring fishery. In Bohuslän, large quantities of herring would migrate to the Swedish Skagerrak coast for periods of many years to overwinter near shore. These periods usually lasted 20–50 years but were separated by periods of 50–70 years. The dates of the four most recent periods are precisely known (Table 1), but five other periods are also known to have occurred although their timing is less precisely known (Alheit and Hagen 1997, 2001). Hence, unlike what is known about the Øresund herring fishery, the Bohuslän fishery has been characterized by periods of exceptionally strong and weak abundance for roughly 1000 years.

The magnitude of the Bohuslän fishery was large, at least by medieval – early modern standards, and appears to have greatly exceeded the medieval herring fishery in the Øresund during the 18th century. Most of the catches for both fisheries were made at the shore using beach seines. Catches at Bohuslän exceeded 216 000 t in 1895–1896 and reached 270 000 t during a fishing season in the 18th century (Lindquist 1983; Alheit and Hagen 1997).

Table 1. Occurrence and duration of Bohuslän herring periods (see also Alheit and Hagen (1997, 2001)).

Period	Date
1	End of 10th century – early 11th century (ca. 970–1120)
2	End of 11th century – early 12th century (ca. 1110–1130)
3	End of 12th century – mid-13th century
4	End of 13th century – 1307–1330
5	Mid-15th century
6	1556–1589
7	1660–1680
8	1747–1809
9	1877–1906

Herring periods had a significant economic and social impact on the towns of the Bohuslän region, which prospered considerably during these periods (Jahnke 2000; Alheit and Hagen 2001; Holm and Bager 2002). As a result of the changes in the local economies, the herring periods were recorded in documents such as sagas, chronicles, and tax reports. This information has been extremely valuable in documenting the occurrence, duration, and magnitude of the Bohuslän herring periods (Alheit and Hagen 1997, 2001; Corten 1999).

The origin of the Bohuslän herring is believed to be from the North Sea, rather than from the Baltic Sea. Investigations of the remnants (skeletons and vertebrae of herring) from train oil factories of the 18th century have shown that herring caught and processed in the Bohuslän area had meristic characters similar to herring from the North Sea but different from herring found in other areas such as the Baltic Sea or Norwegian Sea (Höglund 1978; Alheit and Hagen 1997).

The occurrence of the Bohuslän herring periods has an oceanographic–climatic basis and is related to the distribution and migration of North Sea herring farther into the Skagerrak (Alheit and Hagen 1997; Corten 1999). Alheit and Hagen (1997) have shown that the last seven herring periods occurred during periods of cold climate as represented by a winter severity index (Lamb 1977). As summarized by Alheit and Hagen (1997), climatic and hydrographic conditions during the Bohuslän periods were characterized by cold water in the North Sea – English Channel – Skagerrak, increased ice cover off Iceland and the Baltic Sea, reduced westerly winds, and a minimum frequency of southwesterly winds over England. These climatic and hydrographic conditions represent the negative phase of the NAO. However, the ecological mechanisms that cause North Sea herring to change their migration and distribution patterns in response to fluctuating oceanographic conditions are not known. For example, did the migration patterns change in response to changes in ocean currents or in response to food-web changes? Or did distributions change in response to presence of predators (e.g., marine mammals), which were abundant in the region (see below)? These issues cannot be clarified at the present time.

Interestingly, there was a major herring fishery also in the Limjord, northern Denmark, during most of the 1500s and 1600s. This fishery also had a significant impact on the regional economy (Poulsen 2001) and is therefore well documented in historical Danish archives; some of the documentation includes multi-annual and even multi-decadal

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trap landings by individual fishermen. A joint investigation of the scientific and historical relationships between the Limfjord and Bohuslän fisheries could therefore be fruitful. Such an investigation could eventually be expanded to include the herring fisheries in the North Sea, which are well documented, particularly in the Dutch archives.

Questions related to the Bohuslän herring fishery in the Skagerrak

Are there additional sources to check the reliability of the first five Bohuslän periods? Do the extant customs rolls of the 16th century permit a reconstruction of fishing effort and total catches? How did this fishery affect herring fisheries elsewhere in the region (e.g., Limfjord, North Sea), and vice versa?

The historical harbour porpoise catch in the Danish straits (ICES Subdivisions 22–23)

Harbour porpoises (*Phocoena phocoena*) historically have bred and mated in the Baltic Proper (ICES Subdivisions 25–29) and preyed on all fish species opportunistically. In fall, they emigrated through the Danish straits (Subdivisions 22–23) in pursuit of prey (e.g., herring) or to avoid heavy ice conditions. Porpoises returned to the Baltic in spring, possibly in response to prey (herring) migrations (Møhl-Hansen 1954; Schulze 1996).

The seasonal migrations of the harbour porpoise (Kinze 1995) through the Danish straits gave rise to a targeted hunt in the northern part of the Little Belt (Subdivision 22) since at least 1593 and also at the adjacent Skærbæk in Jutland (Kinze 1995) for a short period during the second part of the19th century. The exploitation took place during the winter months, and catch seasons normally lasted from 11 November – 2 February.

For the 19th century, catch statistics are available or could be calculated for the seasons 1819–1820 to 1899–1900. Between 1790–1791 and 1818–1819, catches were essentially nil, whereas for the seasons 1715–1716 to 1789–1790, correlations between few available catch statistics and an almost complete record of tithe payments allow a crude estimation of the catch level (Fig. 2).

A second important catch site was found in north Sealand (Denmark), yielding about one-third of the catch in the Little Belt. This catch also originates in the early 1500s and continued until ca. 1945; the seasonal catch took place from March to May. Minor catch sites also existed elsewhere in Subdivision 22 and in western Sweden (Kinze 1995). During the period from 1900 to 1950, the exploitation was rather low apparently until the introduction of monofilament nets in commercial fisheries. These led to high rates of incidental catches of harbour porpoises.

Fish, fishing, and marine mammals in the eastern Baltic (ICES Subdivisions 25–32)

Fish and marine mammal communities

The three most important fish species in the central and eastern Baltic Sea (i.e., areas east of the island of Bornholm, ICES Subdivisions 25–32) are cod, herring, and sprat, and all three species are exposed to heavy fishing pressure. Their dynamics (Jarre-Teichmann et al. 2000; Köster et al. 2001) and those of their major prey, crustacean copepods (Dippner et al. 2000; Möllmann et al. 2000), are governed by external physical forcing, biological interactions (Sparholt 1994; Köster and Möllmann 2000*a*), and anthropogenic influences, particularly fishing activities and habitat alteration (Alheit and Hagen 2001). Other fish species (e.g., salmon (*Salmo salar*), flounder (*Platichthys flesus*), gobies (Gobiidae)) are present but their biomass is much lower. Cod is the top predator (aside from man) and herring and sprat are both prey (Sparholt 1994) and predators (Köster and Möllmann 2000*a*) of cod during different parts of its life history. In addition, herring are predators of sprat (Patokina and Feldman 1998).

Historically (i.e., ca. 100 years ago), this picture was quite different because the abundance of these fish species was much lower (Thurow 1997, 1999). Both the landings (Fig. 3) and abundance of cod, herring, and sprat have increased substantially during the 20th century (Bagge et al. 1994; Thurow 1997), although catch data and other biological information required for a reliable reconstruction are often poorly known, particularly before the 1950s (Thurow 1997, 1999). This means that abundances in the early 1900s may be less certain than those in more recent decades.

The marine mammal community is presently small and composed of grey (*Halichoerus grypus*), ringed (*Phoca hispida*), and harbour seals (*Phoca vitulina*) and a remnant population of harbour porpoise (Kinze 1995; Harding and Härkönen 1999). In addition, there are irregular occurrences of 19 other cetacean species (Kinze 1995; Kinze et al. 1998, 2001) in various parts of the Baltic. Seals and harbour porpoise were much more abundant in the early 1900s than they are today (Elmgren 1989; Harding and Härkönen 1999).

The role of marine mammals as predators on fish was therefore much more important than today (Elmgren 1989; Thurow 1997, 1999). Fish consumption rates by marine mammals were probably 320 000 t in 1900 but only 6000 – 10 000 t in 1980 (Elmgren 1989). The inversely related changes in abundance of marine mammals and the three main fish species of the Baltic suggests that predation may have been an important factor regulating abundance of fish species in previous centuries. However, other (environmental) factors may have contributed to the increase in fish biomass (see below).

Cod in the eastern Baltic

The cod stock in the eastern Baltic has been exploited intensively only since the early 1950s. Before this time, the cod fishery is reported to have been very small and may not have reflected stock abundance accurately; landings increased from <10 000 to >400 000 t during the 20th century (Sparholt 1994; Fig. 3). This increase in landings is partly due to increases in fishing effort and changing technology (Bagge et al. 1994). Analytical assessments of stock abundance since the mid-1960s by ICES working groups have shown that abundance (expressed as tonnes of spawning adults) has fluctuated widely since the mid-1960s with a peak of ~800 000 t of spawning biomass in the early 1980s and a low of ~90 000 t at the present time.

Information about cod abundance before 1950 is scarce, partly because the fishery was not fully developed (Kändler and Thurow 1959; Rutkowicz 1959; Bagge et al. 1994). In the 1930s, Polish fishermen complained of low cod catches compared with those in the 1880s and 1890s (Sparholt

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Fig. 2. Historical catch of harbour porpoises at two sites in the northern Little Belt (ICES Subdivision 22; Fig. 1), Denmark, during winters between 1715–1900 (Kinze 2000). All catches were made in winter; in the figure, years are labelled according to winter (e.g., 1716 is the winter 1715–1716).

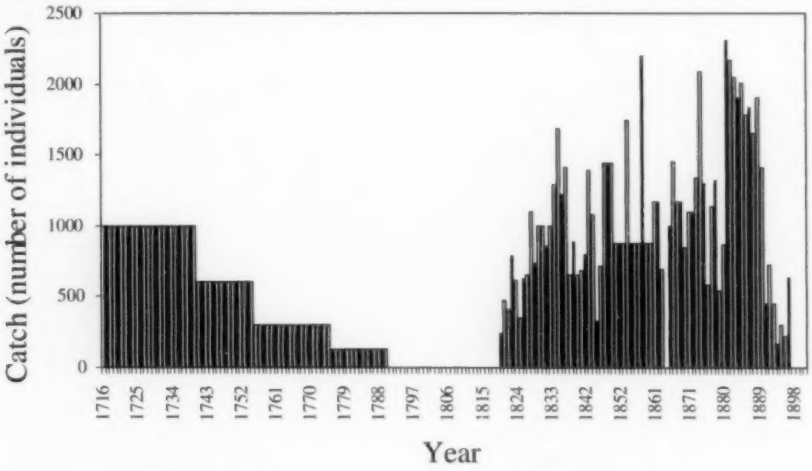
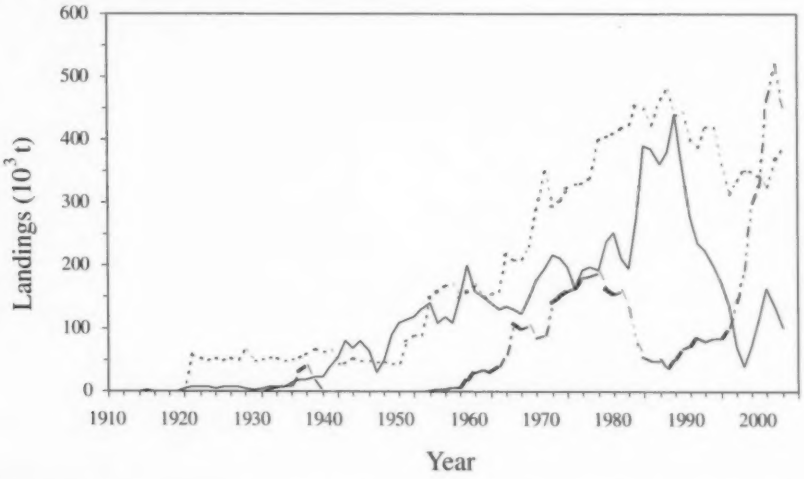


Fig. 3. Commercial landings of cod (solid line), herring (dotted line), and sprat (chained line) in the Baltic Sea (ICES Subdivsions 22–32) during the 1900s as reported to ICES (Sparholt 1994; ICES 2001).



1994). Other anecdotal evidence suggests that a fishable stock existed at least as far back as the 1400s (Otterlind 1984). The evidence used by Otterlind was based on taxes paid to the Swedish King and on occasional natural history records of the presence of cod in some nearshore waters. Archaeological evidence (buried cod bones) from Bornholm (Denmark) and the east coast of Sweden shows that cod were captured during the early medieval period (11th century AD) and the 6th–7th centuries AD (Enghoff 1999).

One recent quantitative reconstruction of cod biomass in the Baltic suggests that cod biomass was only 20 000 – 50 000 t in the entire eastern Baltic during 1900–1930 (Thurow 1997, 1999). This estimate is less than half of the lowest observed biomass since 1966 (ICES 1999) when fishing effort has been very much higher than in the early 1900s. The trend in abundance during the 1900s raises important questions. In particular, has the stock had similar rises (and decreases) in previous centuries, and if so, what were the causes for these changes?

The reconstruction itself relies on many assumptions relating to both the fishery (e.g., unreported landings; changes in fishing technology, fishing skills, and gear deployment) and levels of predation by marine mammals (Thurow 1997, 1999), and therefore, it may be uncertain. The low biomass in the early 1900s is particularly striking relative to ICES assessments of the stock during recent decades. Nevertheless, the reconstruction is consistent with quantitative fishing attempts with otter seines (beginning of 1900s) and steam trawlers (1914 and 1917), which showed that the stock was very small (Meyer quoted in Jensen (1954)). Other colleagues indicate increases in catch per unit effort during the 1930s and 1940s (Jensen (1954) and references to ICES

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Annales Biologiques reports therein) and increases in catches at the northerly limit of cod distribution in the Baltic (Gulf of Bothnia, Hessle 1947). These observations suggest that the stock biomass did indeed increase greatly during the early decades of the 1900s.

There is also evidence from ichthyoplankton surveys that cod abundance and egg production may have increased during the 1940s as a result of the increase in stock abundance. Concentrations of cod eggs in the Bornholm Deep (Fig. 1) during the 1920s–1960s were relatively stable (mean abundance = ~30 m^{-2}; Fig. 4), although there is a large gap in the data series from the late 1930s to 1954 (MacKenzie et al. 1996*a*). However, a recent compilation of historical Polish egg abundance data measured in a neighbouring spawning area to the east (Gdansk Deep; Fig. 1) showed that egg concentrations during 1947–1949 and 1951 were 50–280 m^{-2} (Karasiova 1999). This spawning area has had less favourable environmental conditions for egg survival during the years 1954–1996 than the Bornholm Basin (MacKenzie et al. 2000). However, the high concentration of cod eggs in the Gdansk Basin during the years 1947–1949 and 1951 compared with abundances in the Bornholm Basin in previous years is consistent with both an increase in stock size and an improvement in hydrographic conditions that occurred after the major inflow of saline water to the Baltic in 1947 (Alander 1952; Dementjeva 1959).

The potential contribution of historical research to Baltic cod biology

It will now be proposed that a combined historical–scientific approach can help clarify the development of the stock. This approach could resolve and contribute time series of landings and effort data for long periods when changes in effort and gear type were relatively small. If this is possible, then changes in catch per unit effort could be interpreted relative to socioeconomic (e.g., demand–supply relationships) and biological factors (e.g., changes in abundance, migration patterns, or distribution) as has been done in other areas (Hutchings and Myers 1995).

An ongoing examination of Danish records from Bornholm and the present-day southern Swedish province of Scania (Holm and Bager 2002) has shown that there was a viable cod fishery during the late 1500s and early decades of the 1600s and again between ca. 1750 and 1830. This information is, however, only a small portion of that which is available in both Denmark and other countries and which could be used to develop a full interpretation of fishing activities around the Baltic (Baltic-HMAP 2001).

Nevertheless, the material recovered to date (e.g., numbers of cod paid as tax by individual fishermen to local authorities; Holm and Bager 2002) demonstrates the potential for estimating landings and effort indices. These indices could then be compared with other environmental information to investigate whether abundance and landings co-varied with environmental variables (e.g., sedimentary evidence of inflows; see below). However, this potential can only be met if further historical information about the tax accounts (e.g., constant proportion of landings, or per fisher) and fishery (e.g., gear types, number of fishers and boats, etc.) become available from as many sources around the Baltic as possible.

One parameter to be considered when evaluating the historical fisheries of the Baltic is the allocation of fisheries to different countries during the medieval and early modern period. German towns at this time had the privilege to catch herring but not cod in the Baltic, Øresund, and Skagerrak. Hence records of cod landings will not appear in the historical records of the Hanseatic League but might appear in the archives of other countries such as Denmark, Sweden, and Finland.

Museum and fish processing records might also contribute to understanding and documenting periods of high and low cod abundance. We now know that cod cannibalism in the Baltic is more common when the stock is very abundant (Sparholt 1994). There is also historical evidence that cod cannibalism occurred in the White Sea in the 1800s (Baer 1853). Documentation of cod cannibalism in the Baltic from historical records would be strong evidence that cod was extremely abundant during some periods. Similarly, increases in landings of cod in the northerly areas of the Baltic (Gulf of Finland, Gulf of Bothnia, Hessle 1947) would indicate an increase in geographic distribution of the stock that occurs when the stock becomes very abundant (Aro 1989; Bagge et al. 1994). This increase in distributional area would reduce density-dependent competition for food and habitat in the southern areas (e.g., Subdivisions 25–26). Alternatively, the range of the stock might spread northwards as a direct consequence of an increase in marine habitat area caused by major inflows of salt water.

Questions related to the potential contribution of historical research to Baltic cod biology

Why is the Baltic cod fishery less predominant than the herring fishery in reports of medieval and early modern societies—was there a cod fishery that has not been fully documented, or were cod rare in the Baltic at that time? If cod was abundant in the Baltic, why was it not exported to southern Europeans (e.g., Portuguese, Basques, French, Spaniards), who otherwise began travelling across the Atlantic to obtain supply? Could not the same open-sea harvesting technology used in those areas be applied to cod in the Baltic? What cod taxes were paid in previous centuries in Baltic countries and when, where, and why were they implemented? Did the Hanse League obtain all of its cod from the North Sea (England) and Norway (Bergen), and if so, why not from the Baltic? What other evidence (e.g., archaeological) exists of a cod fishery in the Baltic in the last 500 years? Is the existing 20th century reconstruction of cod biomass and seal predation representative? Was Baltic cod abundance ever >50 000 t before 1900?

Effects of marine mammal predation on cod abundance

Elmgren (1989) has estimated the fish consumption by marine mammals in the Baltic to be ca. 320 000 t in 1900 and 6000 t in 1980. Unfortunately, the contribution of different fish species to this consumption estimate is not precisely known (Elmgren 1989; Thurow 1997). Nevertheless, this fish consumption estimate suggests that predation might have been a major factor limiting cod abundance: seals were considered to be a competitor for fish by Baltic fishermen after the end of the 19th century (Harding and Härkönen 1999). Notably the trends in reconstructed cod abundance

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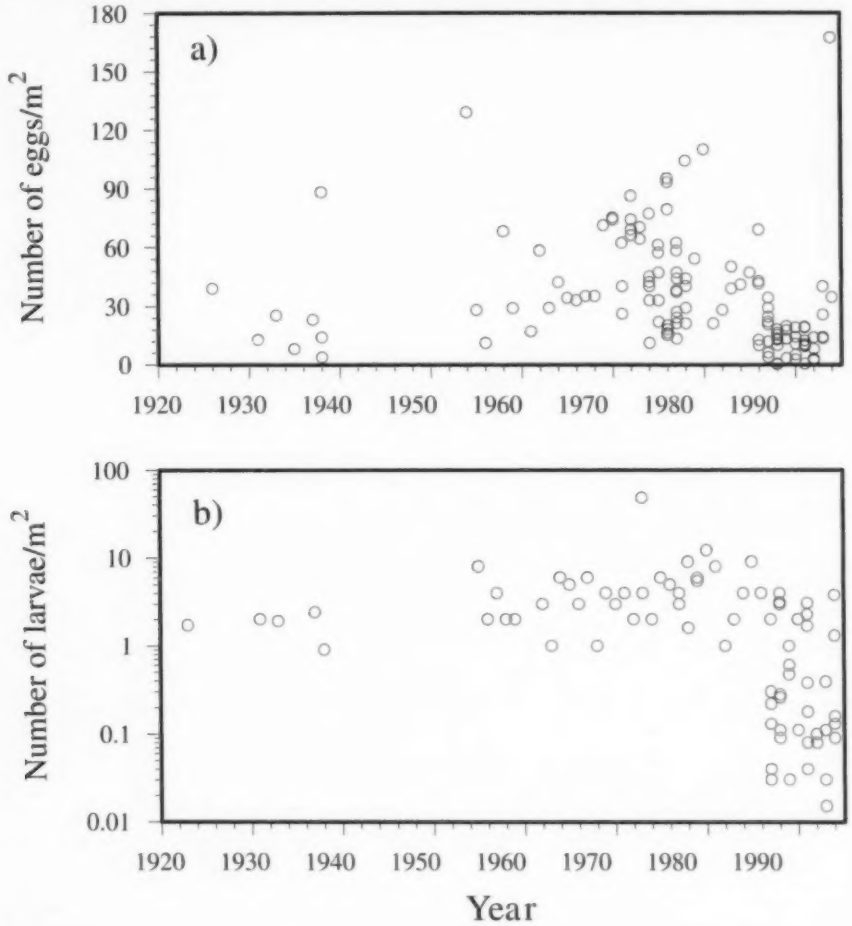
Fig. 4. Abundances of cod eggs and larvae in the Bornholm Basin, Baltic Sea (ICES Subdivision 25; Fig. 1), as measured by different authors (Plikshs et al. 1993; Wieland 1995) during the 20th century (MacKenzie et al. 1996*a*).



(Thurow 1997, 1999), landings (Sparholt 1994), and catch per unit effort (Hessle 1947; Jensen 1954) in the early part of the 1900s are opposite to the trends in the seal and harbour porpoise abundances. However, it is not clear from this evidence alone whether seal and porpoise predation on cod was sufficient to limit cod abundance because the species composition of the diets in the early 1900s is not known. In addition, as noted by Thurow (1997), the large increase in cod, herring, and sprat in the Baltic began only when the seal population was reduced to 20% of its 1900 level. If predation was the main factor suppressing cod abundance, the stock might have been expected to increase earlier. Other factors may have contributed to the increase in cod abundance during the 1900s (e.g., changes in hydrographic conditions; see below).

Historically, seals were very abundant in the Baltic, and sealing formed the basis of many local economies in the northern regions: during 1300 to 1800, the Baltic was the most important producer of seal oil in Europe (Harding and Härkönen 1999). The abundance of grey and ringed seals in the Baltic in the early 1900s was 80 000 – 100 000 and 170 000 – 220 000, respectively; abundances are much lower now (Harding and Härkönen 1999). Harbour porpoises were also much more abundant in the 1700s and 1800s than in the 1900s. Kinze (1995) provided a crude estimate of 25 000 porpoises for the original size of the population in the Baltic Proper (ICES Subdivsions 25–29).

These large populations of opportunistic predators would have required and consumed large amounts of fish, which included species such as cod, herring, and sprat (Lindroth 1962; Källquist 1974). Historical records of seal and porpoise stomach contents could help clarify the extent of predation on cod and other fish species. Such information could be available in records from museums and seal-processing companies.

Questions related to effects of marine mammal predation on cod abundance

What role did seals and porpoises have on Baltic economies (trade in fat, oil, skins, meat?) in the last 4–5 centuries? How large were the seal hunt and the seal and porpoise populations in previous centuries? How important were seals

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and porpoises as cod predators in the Baltic compared with their influence on other cod stocks? Is there evidence from cod, seal, and porpoise stomach contents that cod cannibalism and predation was common in previous times, thereby indicating high cod abundances?

Effects of environmental factors on cod abundance

The abundance of cod in the eastern Baltic depends partly on environmental conditions (Plikshs et al. 1993; Jarre-Teichmann et al. 2000; Köster et al. 2001). The main environmental process presently known to affect cod recruitment is the concentration of oxygen at depths where eggs are neutrally buoyant. Unlike all other cod populations, cod eggs in the eastern Baltic are neutrally buoyant in deep water below a permanent halocline (Bagge et al. 1994; Vallin et al. 1999); cod eggs in areas outside the Baltic float near the surface (upper 20–30 m) where oxygen conditions are sufficiently high to enable normal development.

Oxygen concentrations in the deep layers of the Baltic decrease during periods between major inflows of oxygenated, saline water from the North Sea (Matthäus and Schinke 1999). The reduction in oxygen concentration is frequently sufficient to prevent cod eggs from hatching (Wieland et al. 1994). As a result, the layer of water in the water column that allows cod eggs to hatch depends both on salinity (and therefore egg buoyancy; Vallin et al. 1999) and oxygen concentration. Low salinity water also prevents successful fertilization of cod eggs (Vallin et al. 1999). Oxygen concentrations and salinity therefore are important factors determining recruitment in this cod population (Plikshs et al. 1993; Jarre-Teichmann et al. 2000; Köster et al. 2001). Hydrographic data can therefore be used to derive indices of water volumes that allow successful egg fertilization and hatching (e.g., Fig. 5).

The inflows of North Sea water that renew oxygen concentrations and increase salinities in deep layers of the Baltic depend on particular wind conditions and are therefore at least partly climatically dependent (Schinke and Matthäus 1998). Major inflows (i.e., those sufficiently strong to renew oxygen conditions in the eastern part of the Baltic) occur mainly in the fall–winter period (Schinke and Matthäus 1998) and have been recorded since 1897 (Fig. 6). In the period from 1897 to 1977, events occurred on average almost annually, but since 1977, inflow frequency has declined. During 1982 and 1993, there were no major inflows to the eastern Baltic (Fig. 6), and this situation contributed to the rapid decline of the stock in the same time period.

The stock is therefore sensitive to weather- and climate-driven inflows of North Sea water that allow successful egg fertilization and hatching. One might hypothesize that the increase in cod abundance during the 20th century may have been due to an overall improvement in the oxygen conditions in deep water mediated by an increase in inflows of North Sea water. Thurow (1999) has suggested that much of the increase in stock abundance during the 1930s can be explained by good oxygen conditions in the 1920s and 1930s and that similar conditions have not occurred since then. There is strong evidence to support this suggestion. Scientists at the time documented large increases in salinity during the 1930s and especially the 1940s (Dementjeva 1959). We also know that moderate and strong inflows of North Sea water were frequent and regular during those decades (Schinke and Matthäus 1998; Fig. 6). These observations demonstrate that the hydrographic conditions were adequate for producing strong year-classes and probably were a factor responsible for the high egg abundances during the late 1940s (Karasiova 1999).

However, the hydrographic conditions in the first two–three decades of the 1900s were also probably good for strong cod year-classes. The time series of major inflows to the Baltic shows that these events were especially frequent and strong at the turn of the century, 1907–1915, and 1921–1927 (Fig. 6). Despite good hydrographic conditions, reconstructed cod abundance was extremely low at this time (Thurow 1997, 1999). Assuming that the reconstruction is representative, this pattern demonstrates that good hydrographic conditions are a necessary but not sufficient factor for strong year-classes. The small cod stock in the early 1900s must have been due to other factors, such as predation by marine mammals on juvenile cod (Thurow 1997, 1999) and predation by herring and sprat on eggs and larvae (Köster and Möllmann 2000*a*). Although temperature is a factor that influences cod recruitment in many other areas (Brander 2000), its effect on cod in the Baltic is probably obscured by the effects of salinity and oxygen.

Questions related to effects of environmental factors on cod abundance

If cod was abundant in previous centuries (i.e., >50 000 t, the biomass in the early 1900s; Thurow 1997, 1999), what environmental conditions (e.g., frequent inflows, exceptionally cold or warm temperatures) prevailed during these periods? Can environmental indices affecting cod abundance be extended back several centuries?

Synthesis: cod – marine mammals – environment

The estimated cod biomass in the central Baltic has increased greatly during the 1900s. A key question is how long will the stock remain at its late 20th century level (mean spawning stock biomass = 315 000 t; 1966–1998; ICES 1999)? This question cannot be answered with precision because of the dependence of year-class strength on inflows of saline, oxygen-rich water and probably also predation by herring and sprat. However, given the rapid fall of the stock since the early 1980s, it is conceivable that under specific circumstances the stock could quickly collapse further to its 1900 level (20 000 – 50 000 t). The recipe for collapse seems obvious: infrequent inflows, high fishing mortality, and high predation by herring and sprat. But is it likely that the stock could collapse to such low levels again, even if fishing were stopped completely as the decline progressed?

An answer to this question can potentially be obtained by examining the historical accounts for evidence of fluctuations in previous centuries; the material available for doing this task exists in various institutions around the Baltic. The question that needs to be addressed is did cod landings and abundance vary as much in previous centuries and did the fluctuations coincide with variations in potentially measurable entities (e.g., predator abundances, major inflows).

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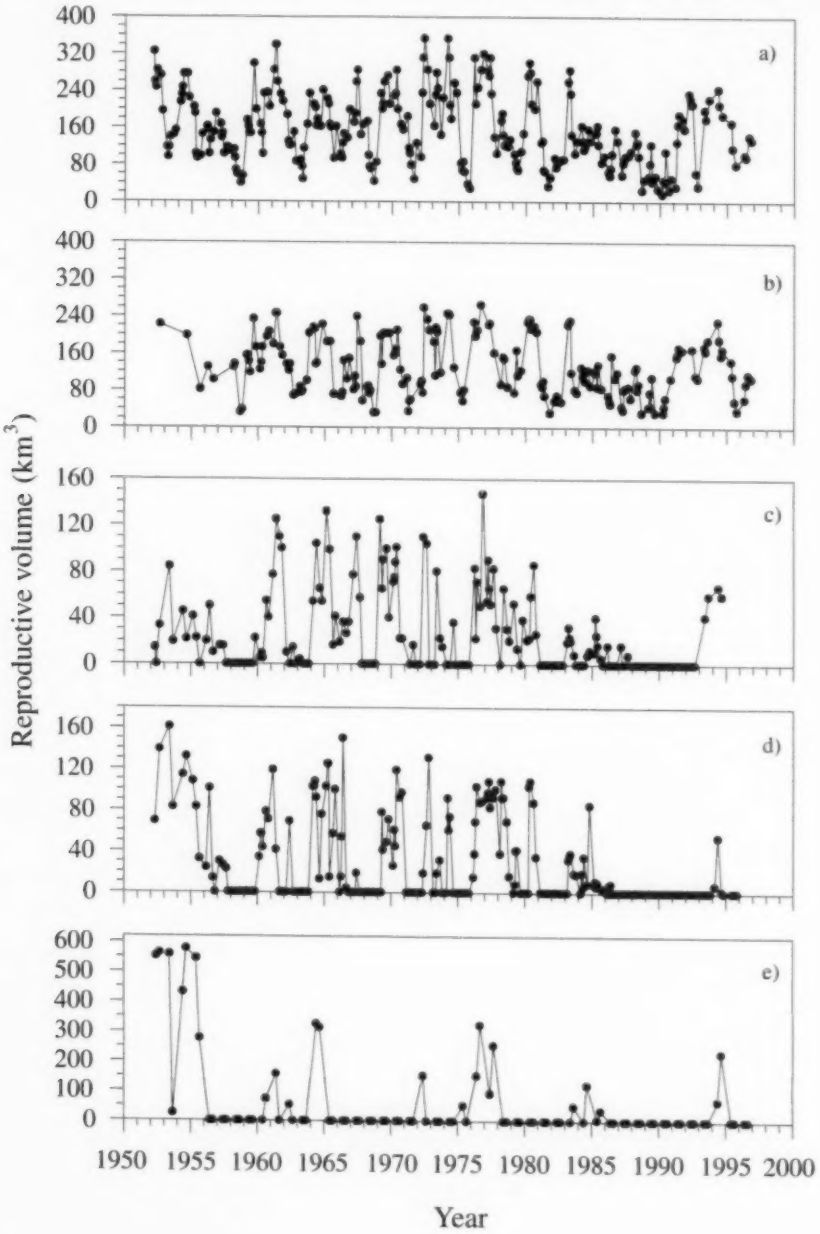
Fig. 5. Volumes of water in the main cod spawning areas of the eastern Baltic (ICES Subdivisions 25–32; Fig. 1) where salinity and oxygen conditions allow successful fertilization and hatching of cod eggs. (*a* and *b*) Volumes in the Bornholm Basin; (*c*–*e*) volumes in the Gdansk, southern Gotland, and central Gotland Basins. Estimates for the Bornholm Basin (*a*, *b*) are derived respectively from Latvian (Plikshs et al. 1993; Plikshs 1996) and international (i.e., ICES; *b*) data sources (Hinrichsen and Wieland 1996). Estimates for the other areas are derived from Latvian data (Plikshs et al. 1993; Plikshs 1996). See MacKenzie et al. (2000) for details.



Herring and sprat fisheries in the Baltic (ICES Subdivisions 22–32)

The stocks of herring and sprat in the eastern Baltic have increased substantially in the 2nd half of the 1900s (ICES 1999), although the herring stock has been decreasing almost annually since 1974 (ICES 1999). The biomass of sprat increased since the early 1980s partly because of the reduction in predation by cod (Sparholt 1994), whose abundance has fallen (ICES 1999) during the same time period. In general, cod predation on herring and sprat is important for the

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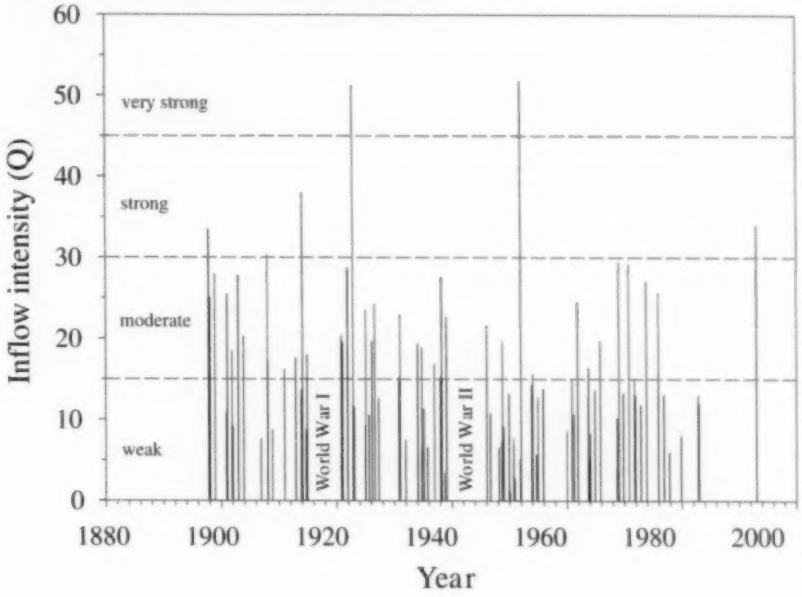
Fig. 6. Occurrences and intensities of major inflows of North Sea water into the central Baltic Sea (ICES Subdivisions 25–32; Schinke and Matthäus 1998). Inflow intensity is estimated from salinity measurements at light vessels located in the western Baltic (near border of Subdivisions 22 and 24) and near the Øresund (near border of Subdivisions 23 and 24). Data reproduced with permission of Dr. W. Matthäus, Baltic Sea Research Institute, Warnemünde, Germany.



abundance of all three stocks (Sparholt 1994). In addition, herring and sprat are predators on cod eggs and larvae (Köster and Möllmann 2000*a*). The herring in the Baltic traditionally had two spawning components (spring and fall spawners), but now only spring spawners are left (Parmanne et al. 1994). The decrease of the fall spawners is believed to be due to fishing and to anoxia in herring spawning grounds (Parmanne et al. 1994).

Historically, it is believed that these stocks were also low in the early 1900s but increased after eutrophication (Thurow 1997). These stocks remained low until the mid-1940s, long after the point at which predation by marine mammals had fallen. In other words, the onset of the increase in the pelagic stocks occurred well after the decrease in seal and porpoise predation (Thurow 1997), and the relative timing of these events led Thurow (1997) to suggest that eutrophication was the main reason for the increase in herring and sprat abundance.

The herring fishery yield in the Baltic Proper (ICES Subdivisions 25–29) is presently several-fold larger than that of the medieval Øresund fishery. Whether the yield was also larger historically is unknown and perhaps not likely, given that medieval – early modern fishermen concentrated their efforts in the Øresund area (Jahnke 2000; Holm and Bager 2002). If, however, further research could document a fishery for herring (or sprat) in the Baltic Proper, its landings and biomass could be compared with those in the Øresund and Bohuslän areas to evaluate whether fisheries in these regions co-vary with other European herring and sardine fisheries (Alheit and Hagen 1997). Historical records of herring and sprat landings and stomach contents of their predators (marine mammals, cod, salmon) could also be used to examine predator–prey interactions at century scales.

Environmental processes affecting the herring and sprat stocks are unclear (Parmanne et al. 1994). Herring recruitment in the Gulf of Riga increases with temperature (ICES 1999), but it is not known whether a similar relationship exists for other Baltic herring stocks (Parmanne et al. 1994; ICES 1999). Growth rates of herring during the 1980s–1990s have decreased (even though herring abundance has also decreased; Flinkman et al. 1998; Cardinale and Arrhenius 2000) because of climate-related changes in availability of preferred prey sizes and species (Flinkman et al. 1998; Dippner et al. 2000; Möllmann et al. 2000).

Sprat recruitment is positively correlated with temperature both in individual subdivisions (Ojaveer 1998) and in the entire Baltic (Subdivisions 22–32; B.R. MacKenzie and F.W. Köster, unpublished data). During the years 1973–1999, sprat recruitment for the whole Baltic increased significantly with increases in temperature and was correlated with winter ice and NAO indices (B.R. MacKenzie and F.W. Köster, unpublished data). These relationships could help infer how abundances and the species composition of the fish community varied under previous warm or cold periods.

Questions related to herring and sprat fisheries in the Baltic (ICES Subdivisions 22–32)

What evidence exists for herring and sprat fisheries in the eastern Baltic prior to 1900? Did the herring or sprat fisheries co-vary with the herring fishery in the Øresund, Bohuslän, or other European areas? What were the relative roles of climate variability and cod, seal, or porpoise predation on his-

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Table 2. Summary of some sources of long-term hydrographic and climate datasets for the Baltic region.

Type of data	Years of coverage	Source
North Atlantic Oscillation	1824–present	Jones et al. 1997 and http://www.met.rdg.ac.uk/cag/NAO/index.html
Daily air temperatures, Bornholm	1880s–present	Danmarks Meteorologiske Institut reports
Daily temperature, salinity measurements at three depths and eight stations in Danish waters	1900s–1980s	Sparre 1984
Records of inflow intensity and duration	1897–present	Schinke and Matthäus 1998
Harbour opening, Riga, Latvia	1500s–1950s	Lamb 1977
Ice-cover data for entire Baltic Sea	1720–present	Seinä and Palosuo 1996 and updates at http://ice.fmi.fi/main_uk.html
Ice volume thickness in southwestern Baltic	1879–present	Koslowski and Loewe 1994

torical herring and sprat fisheries? Did the herring or sprat fishery oscillate inversely with the cod fishery in the eastern Baltic (predator–prey interactions)? Are there long-term variations in the herring or sprat fisheries that coincide with climatic and hydrographic variability? Given the strong predator–prey interactions between cod and both herring and sprat, why did all three stock levels rise so much during the 1900s?

Detecting long-term environmental variability in the Baltic Sea

Attempts to understand long-term biological changes in the Baltic Sea must also include descriptions of past environmental variations. Existing knowledge suggests that the most important environmental factors for the biota of the Baltic Sea are salinity, oxygen concentrations, temperatures, and eutrophication. However, measurements of most of these parameters are available only for the last century. These data are reviewed periodically (Helcom 1996).

Estimating environmental conditions farther back in time requires proxy data series and sedimentary evidence. For example, data series and relationships involving the NAO, ice coverage, and water temperature should allow reconstruction of temperature conditions potentially from the 1500s. Coastal monitoring stations and lightships in the Baltic (Table 2) have recorded water temperatures daily or weekly from the late 1800s – early 1900s to the present (Sparre 1984), and at least three annual ice coverage – winter severity indices exist for the Baltic from the 1500s (date of final opening of Riga harbour; Lamb 1977), 1720 (Fig. 7; Seinä and Palosuo 1996), and 1879 (Koslowski and Loewe 1994). Water temperatures in the Baltic at least in spring and early summer are strongly correlated to winter ice coverage (MacKenzie et al. 1996*b*; B.R. MacKenzie and F.W. Köster, unpublished data) and ice conditions in the Baltic co-vary with the NAO (Koslowski and Loewe 1994; B.R. MacKenzie and F.W. Köster, unpublished data). These relationships, and those including tree growth rings and other data series from northern Europe, could help identify how the "Little Ice Age" influenced fishing and fish stocks in the Baltic and how climate change (e.g., global warming) might influence fish stocks in future.

Sediment cores taken from the deep basins also reveal century-scale variations in environmental conditions and the biota of the Baltic Sea. These analyses could be particularly useful for identifying major inflows from the North Sea. First, inflows over the last 1000 years have been characterized from chemical composition of the sediments, which is an indicator of hydrographic variability associated with saltwater inflows (Neumann et al. 1997). Second, benthic foraminifera have been found in marine sediments elsewhere in the Baltic. Because they are indicators of hypoxic conditions (Bernhard and Sen Gupta 1999), they might be useful indicators of inflow events in the Baltic. However, their utility has not yet been investigated for this purpose. Third, the presence of laminae in sediments is an indicator for longer periods of anoxic conditions (Jonsson et al. 1990).

Other biological evidence of inflows could support the paleo-oceanographic findings. For example, sightings of 19 marine mammal species south of the Skagerrak–Kattegat transition area may be indicative of more marine conditions (Kinze et al. 1998). Historical observations of cetaceans in various parts of the Baltic might also indicate occurrence of increased salinities in the Baltic.

Paleo-oceanographic studies potentially give us a longer perspective to interpret the consequences of eutrophication on Baltic food webs. For example, although it is believed that cyanobacterial blooms are now more frequent and intense during the post-eutrophication era (Kahru et al. 1994), paleo-oceanographic evidence (e.g., phytoplankton pigment remains) shows that blooms also occurred frequently during the last 8000 years (Bianchi et al. 2000). Eutrophication has reduced silicate concentrations in the Baltic (Conley et al. 1993). In other areas, reduced silicate levels have led to a change in species composition of the phytoplankton community and can change the base of the entire food web (Conley et al. 1993). Whether similar changes have occurred in the Baltic and how these changes affect production at upper trophic levels is not known.

Lastly, paleo-oceanographic studies could reveal direct evidence of the occurrence of fish remains. Such evidence (e.g., preserved scales, otoliths, bones) is most frequently found in deep, permanently anoxic areas of the sea (e.g., off the coasts of California and Namibia; Francis and Hare 1994; IGBP 1999) and have been used to infer changes in fish abundance. Whether fish remains are preserved in Baltic sediments is unclear but the topic deserves to be investigated.

Questions related to detecting long-term environmental variability in the Baltic Sea

How did Baltic fisheries and seal hunts change under dif-

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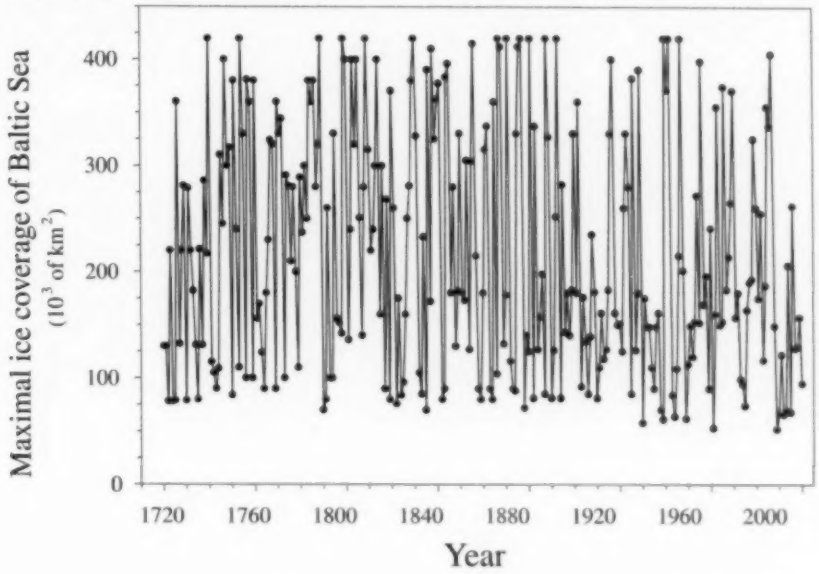
Fig. 7. Annual maximal area of sea ice coverage of the Baltic Sea (Subdivisions 22–32 and Kattegat) from 1720–1995 (Seinä and Palosuo 1996) and 1996–2000 (updates from the Finnish Ice Service available online at http://ice.fmi.fi/main_uk.html).



ferent climate scenarios? Were seasonal fisheries and hunts delayed or shortened and were landings for some species lower in cold periods compared with warm periods? Can a reliable time series of major inflows of North Sea water be constructed from sedimentary evidence? Can such a time series be supported by other biological or environmental information such as porpoise and whale sightings and kills following intrusions of salt water? Can sediment cores indicate changes in taxonomic base of food web production?

A speculative view of past and future variations in upper trophic level biomass in the Baltic Sea

We have been asked by the journal editor "to speculate further," "to be bolder in our interpretations," and "to present some ideas that would not normally make it into the literature" when revising our manuscript. We have done this in several places throughout the text by challenging the reader with specific questions and hypotheses about biological and socioeconomic aspects of fishing and hunting in the Baltic Sea and Skagerrak.

Below, we consider some of the limitations associated with interpreting historical data and how these data could be used to improve our understanding of long-term population dynamics and trophic coupling in the Baltic Sea. We then speculate how the fish and marine mammal biomass in the Baltic Sea might change during the next 100 years, given the implications and inferences possible from the information we already have.

Throughout most of this paper we have encouraged a multidisciplinary search, recovery, and interpretation of historical fisheries and environmental data for the Baltic region. The potential benefits of such an exercise could be very useful and could clarify the past (and future) roles of exploitation, predation, eutrophication, and climate change on fish populations in the Baltic.

The data that will eventually be recovered will be of varying quality depending on factors such as record-keeping practices and the availability of archives. The best type of data that could provide the most information would be several long time series of catch and effort data in consistent units from different locations around the Baltic (see Ravier and Fromentin (2001) for such an example for bluefin tuna (*Thunnus thynnus*) in the Mediterranean). However, given the different and varying administrative and fishing practices around the Baltic throughout the last 500 years, the recovered data will likely have a much different character from the ideal. They will probably consist of broken time series lasting a few years or decades, be expressed in different units, and probably have been obtained with different fishing practices. Some information will be recorded as true catch data, but other data will be fishery taxes (i.e., barrels of fish paid to local authorities as a tax on fishing activity) or "snapshots" of fishing activity based on archaeological evidence. In all cases, it must be recognized that data errors familiar to 21st century fisheries biologists (e.g., misreporting and nonreporting, inadequate reporting coverage) will also apply to the historical data, and that the historical material was not collected with the objective of monitoring population status. As a result, changes in abundance or distribution should be supported with evidence from as many sources as possible.

Differences in data formats will therefore restrict the level of interpretation associated with each dataset, and some effort towards standardisation and calibration of the datasets will be necessary before quantitative analyses can proceed. Nevertheless, these different types of information can be interpreted scientifically (Francis and Hare 1994; Hutchings and Myers 1995; Alheit and Hagen 1997). For example, a

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recent study has shown that mankind at a global scale has been exploiting marine resources to and beyond extinction for 1000s of years (Jackson et al. 2001). We are optimistic that results similar to Hutchings and Myers (1995), Ravier and Fromentin (2001), Jackson et al. (2001), and others can be achieved in the Baltic, but we are aware that such results will require many years of archival research in different countries and that a new collaboration between ecologists, oceanographers, and historians be developed and encouraged. Some effort towards these objectives has begun as part of the History of Marine Animal Populations project (http://core.ssc.erc.msstate.edu/censhmap1.html).

How can the recovered data be used once assembled, standardized, and calibrated? We believe that there are at least five ways that recovered time series and "snapshots" of fish landings and effort data could be used. These applications include exploratory statistical analyses, model development, and model validation and are now outlined briefly.

(1) Comparisons of temporal catch trends within and among species, spatially, and with environmental variables. These comparisons would identify correlations at longer time scales than presently possible and could support existing hypotheses and mechanisms of population fluctuation (see sections above) with independent data.

(2) Extension of age- or size-structured virtual population analyses (VPA) backwards in time. This approach has been conducted for three species in the North Sea (Pope and Macer 1996). If applied to Baltic fish stocks, the approach could be used as an alternative means to estimate stock biomasses (cf. Thurow 1997) and to support results obtained with catch data (see 1). Some catch-at-age information is already available for the pre-VPA period (Thurow 1997), and additional data may be available in archives of fishery research institutes. Compilation of such information could be relatively easy (at least compared with researching medieval archives!) and could enable some VPAs to be extended backwards several decades.

(3) Bioenergetic and multispecies models of population dynamics could be developed and compared to the observed fluctuations in biomass of key species. For example, such models could be used to check whether the large increase in cod biomass in the 1940s and 1950s could have been supported by the existing biomass of prey (i.e., herring and sprat). Are fluctuations in the different species consistent with theories of bioenergetics and predator–prey interactions?

(4) Trophic models of food webs (e.g., Christensen and Pauly 1998) for different time periods could be developed and tested with the longer time series. These models could evaluate the relative roles of changes in primary production (resulting from eutrophication) and marine mammal predation on fish stock dynamics, as well as the time lags between changes in dependent and independent variables. For example, can the increase in herring and sprat biomass in the 1950s and 1960s be explained by the eutrophication-related increase in primary production, or was the increase in clupeid biomass due more to the earlier decline in predation by marine mammals?

(5) Development and extension of ecosystem models (i.e., nutrient–phytoplankton–zooplankton) to higher trophic levels. One current research initiative in the Baltic and elsewhere is the development of process-based coupled biological–physical oceanographic models (Euroglobec 1998). Extending these modelling approaches to higher trophic levels is a challenge. However, even given their structural and parameter uncertainties, such models should be able to detect major changes in fish biomass such as those that occurred in the Baltic Sea during the 20th century. Reliable estimates of fish abundance over long periods of time and for situations of radically different ecosystem structure would provide an appropriate test scenario for validating such models.

Our review of the long-term changes in fish biomass and Baltic ecosystem has identified several potential inconsistencies in our perception of linkages among trophic levels and the timing of the responses of some trophic levels to changes at other trophic levels in the food web. For example, how did the cod stock increase in biomass during the 1940s and 1950s when its principle food supply (herring and sprat) was relatively low? Why did the herring and sprat stocks then increase in the 1950s and 1960s, if the abundance of their main predator (cod) was already quite high? Was this increase limited to areas of the Baltic where cod is not normally present (i.e., northern areas, Gulf of Finland and Gulf of Riga)? If eutrophication is the main reason for the increase in herring and sprat stocks, is there any evidence that growth rates or condition in these stocks increased as the effects of eutrophication propagated up the food web and translated into higher food supplies for herring and sprat?

These apparent inconsistencies could be due to uncertainties and violation of assumptions used in the existing fish biomass reconstructions for the Baltic. However, they could also be real and partly due to weak coupling between trophic levels in the Baltic. Compared with other temperate-boreal marine ecosystems (e.g., North Sea), this ecosystem has few species (Voipio 1981) and its strong horizontal and vertical hydrographic gradients provide refuges (Köster and Möllmann 2000*a*, 2000*b*) from predators (e.g., cod is absent in large areas of the Baltic). These factors perhaps reduce the intensity of predator–prey interactions and could result in delayed reactions of one trophic level to changes in abundances of organisms at other trophic levels.

What will the levels of upper trophic level biomass be in 100 years ? This is of course a difficult question to answer, particularly when our best data series are only 35 years long and do not span the full combination of biological and hydrographic interactions that affect upper trophic level biomass in the Baltic Sea.

Nevertheless, there will be at least two major changes to the Baltic Sea ecosystem within the next century that we know from past observations have affected fish production in the Baltic. First, coupled atmosphere–ocean models predict that global climate change will result in higher air temperatures, more precipitation, and an increase in storm frequency in northern Europe (IPCC 2001). The first two changes will probably lead to warmer sea temperatures and lower salinities in the Baltic. A second, indirect, effect of increased precipitation will be to reduce the impact of major inflows of North Sea water on salinities in the deep layers of the Baltic (Matthäus and Schinke 1999). These effects of higher precipitation could be partly counterbalanced by the increased

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windiness, which could increase the hydrographic exchange with the North Sea. If this occurs, then salinities in the deeper parts of the Baltic would increase. However, the relationship between wind conditions and inflow occurrence is complex (e.g., requiring winds from specific directions, differences in sea levels at different locations around the Baltic), and a simple increase in wind speed or storminess will by itself certainly not increase inflow frequency or occurrence (Schinke and Matthäus 1998; Hinrichsen et al. 2002). We therefore speculate and assume that salinities in the deep and surface layers of the Baltic will be lower in 2100 than at present, given the IPCC model results.

The second major change in the Baltic Sea is an expected long-term decrease (multidecadal to century scale) in nutrient loading to the Baltic, although an increase in the next decade or two might occur (Gren et al. 2000). The decrease is expected especially because of reductions in fertilizer use and fossil fuel consumption. In principle, this decrease should result in lower nutrient concentrations in the Baltic. However, although decreasing, nutrient concentrations in the Baltic may nevertheless remain high (i.e., above pre-World War II levels) because of the expected increase in precipitation associated with climate change and the large reservoir of nutrients in the watershed and in sediments of the Baltic Sea (Helcom 1996; Gren et al. 2000).

How will these changes affect abundances of fish and marine mammals in the Baltic Sea? We would expect that the higher temperatures and lower salinities would be detrimental especially to cod in the eastern part of the Baltic Sea. This prediction is based on the relationships between inflow intensity and frequency, deep-layer salinities and oxygen levels, cod egg survival and fertilization success, and recruitment, which are described in previous sections. Moreover, the higher temperatures will result in higher oxygen consumption rates and therefore reduce oxygen levels in cod spawning areas. In addition, there is evidence from comparative studies that carrying capacity for cod in different parts of the northeast Atlantic decreases with increases in temperature (Myers et al. 2001).

How these changes will affect sprat and herring is presently less clear than for cod. The warmer temperatures should be advantageous for sprat (see previous sections) and also for herring (at least in the northern areas; ICES 1999). The reduction in salinity caused by the increase in precipitation and freshwater runoff will probably slightly reduce the range of sprat in the Baltic Sea but should have relatively little direct effect on herring, which is already distributed throughout nearly all parts of the Baltic and therefore experiences low salinities. However, because both species feed directly on zooplankton, they may be more sensitive to a reduction in nutrient loading (cf. cod) and subsequent decreases in primary and secondary production. Growth rates and possibly survival rates could therefore become food limited, particularly as temperatures (and therefore food demands) rise and the abundance of preferred high-salinity prey decreases with an expected decrease in salinity (Flinkman et al. 1998; Cardinale and Arrhenius 2000; Möllmann et al. 2000). It is unclear whether these nutrient- and salinity-related effects will be large enough to offset the effects of temperature.

Climate change may also influence the abundance of marine mammals in the Baltic, especially seals. Because some species (i.e., Baltic ringed and grey seals) breed on sea ice (Reijnders et al. 1997), milder winters will probably result in a loss of breeding habitat and a shift in breeding location to more northerly areas. Hence, although conservation efforts exist to increase seal and harbour porpoise populations, the effects of these efforts in the case of seals may be partly offset by the loss of sea-ice breeding habitat. If this is the case, then predation rates by seals on fish will probably remain relatively low (at least compared with levels in 1900), particularly in the southern areas.

In short, we anticipate at the present time that sprat and herring will benefit from predicted changes in global climate and regional nutrient loading but that cod will suffer in the eastern Baltic. However, we emphasize that these predictions are indeed speculative and based on incomplete knowledge of key biological and hydrographic processes, including species interactions. In fact, the issue deserves a major investigation and could be partly addressed using some of the modelling approaches identified above (e.g., coupled biological–hydrographic models, extended VPA, sensitivity analyses, etc.).

Conclusions

Baltic fish populations have fluctuated at decadal and even longer time scales. However, the reasons for the fluctuations and the impact of these fluctuations on local economies are only partly known. Forecasting how these populations and the ecosystem will change in future following natural and man-induced perturbations (e.g., changes in climate, nutrient loading, fishing effort) will be difficult without a better understanding of the causes of population fluctuations. The documentation and interpretation of historical fisheries, hunts, and environmental change is one step towards achieving such an understanding. Major gaps in knowledge and even some contradictions about the importance of some Baltic fisheries (e.g., cod, herring in the main Baltic Proper) exist, which deserve further investigation. Addressing some of the issues outlined above via new collaborative configurations could be both insightful and exciting.

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stimulating papers on fish biomass and marine mammal predation in the 20th centuries written by Prof. Dr. Fritz Thurow, a long-retired, but still active, colleague.

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DISCUSSION

Comment: Does spawning stock affect recruitment of New England groundfish? Interpreting spawning stock and recruitment data in New England groundfish[1]

D.J. Gilbert

Brodziak et al. (2001) analyzed stock–recruitment time series for 11 New England groundfish stocks and concluded that their "results provide strong evidence that spawning-stock size affects recruitment." A more careful examination of their results suggests that this conclusion derives largely from their prior assumptions. They carried out Bayesian analyses using six alternative models that predict recruitment. In two of these models, mean recruitment is constant (no stock–recruitment relationship). In the other four, it depends on spawning-stock size, with low stock size causing low recruitment. Each of these models is implicitly given an equal prior probability. From summing the prior probabilities over the possible models, it is apparent that the authors held the prior view that a stock–recruitment relationship is twice as likely as no relationship (4/6 versus 2/6 gives a prior odds ratio of 2.0). The effect of the data is obtained by comparing the posterior odds ratios with the prior odds ratio. In 7 of the 11 stocks, the posterior odds ratio of a stock–recruitment relationship being true is less than the prior (Table 1). For these stocks, the effect of the data is to lessen the authors' prior belief that stock size affects recruitment. However, the data generally contains limited information, with only three posterior probabilities showing substantial change from the prior. For two stocks, the data reduce the odds ratio of a stock–recruitment relationship from 2.0 to below 0.4, and for one stock, the data increase it.

The frequentist contingency table analysis carried out by Brodziak et al. is no more conclusive than the Bayesian analysis. In five stocks, the odds ratios of low recruitment when spawning stock size is low and high recruitment when spawning stock size is high are significantly greater than 1. Brodziak et al. assumed that a stock–recruitment relationship is the only possible explanation for high odds ratios. However, stock–recruitment time series are not independent observations. Any persistent cause of low recruitment will cause spawning-stock size to subsequently fall. When persistently low recruitment occurs, it can cause high odds ratios and give the appearance that low spawning-stock size is causing low recruitment. Low spawning-stock size is one possible cause but exogenous causes are also possible. The inference that high odds ratios are necessarily the result of stock–recruitment relationships is incorrect. The "recruitment states" hypothesis (Gilbert 1997) is a possible model where an exogenous factor causes recruitment to periodically switch between different mean levels. For example, both the frequentist and Bayesian analyses of the Georges Bank haddock (*Melanogrammus aeglefinus*) data suggest that the stock may follow a stock–recruitment relationship. However, examination of the time series of recruitments (fig. 4*f* in Brodziak et al. 2001) shows a sudden switch in the mid-1960s from consistently high to almost invariably low values. A recruitment states model appears to explain these data at least as well as a stock–recruitment model. An odds ratio may differ significantly from 1 under either hypothesis.

I conclude that these New England groundfish data do not provide clear evidence as to whether or not spawning-stock size affects recruitment.

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D.J. Gilbert. National Institute of Water and Atmospheric Research Ltd., P.O. Box 14 901, Wellington, New Zealand (e-mail: d.gilbert@niwa.cri.nz).

[1]Comment on Brodziak, J.K.T., Overholtz, W.J., and Rago, P.J. 2001. Can. J. Fish. Aquat. Sci. **58**: 306–318.

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Table 1. Prior and posterior probabilities (%) and odds ratios of alternative stock–recruitment models under a prior where the odds ratio of a stock–recruitment relationship is 2.00 for 11 New England groundfish (modified from Brodziak et al. 2001).

	No stock–recruitment relationship		Stock–recruitment relationship				
	Constant recruitment		Beverton–Holt		Ricker		
	Uncorrelated errors	Correlated errors	Uncorrelated errors	Correlated errors	Uncorrelated errors	Correlated errors	Odds ratio of a stock–recruitment relationship
	Prior probability (%)						Prior odds ratio
	17	17	17	17	17	17	2.00
Stock	Posterior probability (%)						Posterior odds ratio
Gulf of Maine							
American plaice (*Hippoglossoides platessoides*)	5	31	17	37	1	10	1.78
Witch flounder (*Glyptocephalus cynoglossus*)	18	5	13	3	49	13	3.35
Cod (*Gadus morhua*)	43	20	15	7	11	5	0.59
White hake (*Urophycis tenuis*)	28	8	9	2	50	2	1.78
Yellowtail flounder (*Limanda ferruginea*)	64	17	16	3	1	0	0.23
Georges Bank							
Haddock (*Melanogrammus aeglefinus*)	0	9	0	46	0	45	10.11
Cod (*Gadus morhua*)	32	14	14	8	20	12	1.17
Yellowtail flounder (*Limanda ferruginea*)	0	29	2	42	2	24	2.45
Winter flounder (*Pseudoplueronectes americanus*)	23	8	15	4	40	10	2.23
Southern New England							
Yellowtail flounder (*Limanda ferruginea*)	4	32	13	13	23	16	1.78
Winter flounder (*Pseudoplueronectes americanus*)	0	72	0	27	0	1	0.39

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DISCUSSION

Reply: Does spawning stock affect recruitment of New England groundfish? Interpreting spawning stock and recruitment data in New England groundfish[1]

J.K.T. Brodziak, W.J. Overholtz, and P.J. Rago

We offer this rejoinder to Gilbert's (2002) discussion of our analyses of stock–recruitment data for several New England groundfish stocks (Brodziak et al. 2001). The first point that Gilbert makes—that our choice of prior probabilities affects the calculated posterior probabilities—is of course true of all Bayesian analyses. The prior odds ratio of a density-dependent stock–recruitment model being true over the range of observed data was assumed to be 2.0 in Brodziak et al. (2001) because this value was consistent with our beliefs in the relative credibility of the six stock–recruitment models examined. Based on the results of Myers and Barrowman (1996), an empirical Bayesian analysis could make an alternative argument that the prior odds ratio used by Brodziak et al. (2001) for density-independent stock–recruitment models was too ecologically optimistic. Regardless, we must point out that for 7 of the 11 stocks examined, the posterior odds ratio of a density-dependent stock–recruitment model being true over the range of observed data either increased to above 2.0 or decreased slightly to 1.78 (see table 1 of Gilbert 2002). This fact indicates that our assumed prior odds ratio was consistent with the observed data and also shows that for these stock–recruitment data, density dependence was more likely than density independence in the majority of stocks. Nonetheless, we concur with Gilbert's implicit point that a key step in applying Bayesian analyses to natural resource management issues is selecting public policy priors. Furthermore, we submit that if Gilbert's (1997) "recruitment-states hypothesis" is to be a credible prior belief for a specific system during a specific time period, then data and mechanisms should be presented to conclude that physical oceanographic change implies a change in fish stock dynamics (e.g., Baumann 1998). Unfortunately, Gilbert (2002) offers neither in his discussion.

The second point that Gilbert (2002) makes is that the frequentist odds ratio analyses of Brodziak et al. (2001) are invalid because they do not account for the possibility that Gilbert's recruitment-states hypothesis (or other unspecified mechanisms) could be true for the time period and system examined. This is a seemingly persuasive argument, because if the odds ratio analyses are valid, then any medium- or longer-term changes, such as a regime shift in oceanic conditions, could not have occurred under the implicit assumption that groundfish recruitment strength changes substantially from one average state to another following such a shift. Simply denying that the odds ratio analyses are valid without providing evidence of such a regime shift is a fallacy, however. If changes had occurred in the continental shelf waters off New England during the time period of our investigation, we would expect that important environmental forcing processes, such as water temperature, would have changed substantially in an obvious directional manner. Instead, water temperature anomalies for this region exhibit short-term variation on the order of a few years but do not show interdecadal time trends (Fig. 1). Furthermore, longer-term forcing variables, such as the North Atlantic Oscillation (NAO), appear to have had a limited influence on the dynamics of fish populations within the northeast United States continental shelf ecosystem in comparison to fishing activities (Link et al. 2001). Although oceanographic conditions can substantially affect recruitment of New England groundfish in any given year (see, for example, Polacheck et al. 1992), there is no conclusive evidence of any medium-term change or a sustained regime shift in the continental shelf waters off New England that would support the recruitment-states hypothesis during the time period of our investigation. Thus, Gilbert's rejection of our frequentist odds ratio results is not valid.

We conclude by noting that our assumption that recruitment is constant over the range of observed stock sizes is not equiva-

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J.K.T. Brodziak,[2] W.J. Overholtz, and P.J. Rago. National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, MA 02543, U.S.A.

[1]Reply to comment of Gilbert, D.J. 2002. Can. J. Fish. Aquat. Sci. **59**: 191–192.

[2]Corresponding author (e-mail: Jon.Brodziak@noaa.gov).

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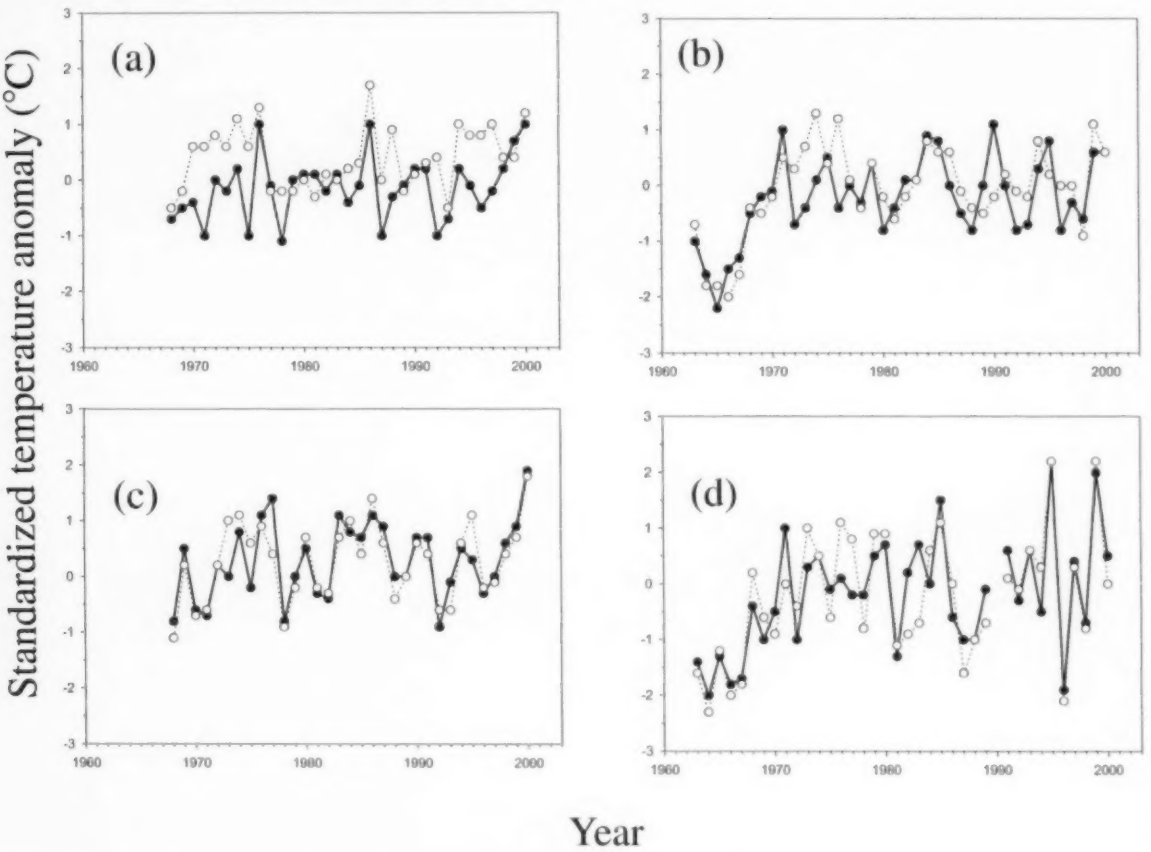
Fig. 1. Standardized surface (solid circles) and bottom (open circles) water temperature anomalies (David Mountain, Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543, U.S.A., personal communication) for the Gulf of Maine during (*a*) spring and (*b*) autumn and for Georges Bank during (*c*) spring and (*d*) autumn.



lent to the hypothesis that "no stock–recruitment relationship" exists. In fact, the assertion that no stock–recruitment relationship exists is devoid of meaning as an alternative hypothesis. Consider the following simple proof. Let S equal the survival rate from egg (Egg) to recruit (R) stage such that $R = S \times \text{Egg}$. Assume that S is completely random on the interval [0,1] and is unaffected by stock biomass. The expected value of recruits is then $E(R) = E(S \times \text{Egg}) = E(S) \times E(\text{Egg})$. Thus, under the assumption that recruitment is driven primarily by stochastic variation in survival rate, recruitment will always be proportional to egg production or some function of stock biomass. The ability to detect the form of dependence may be compromised by high variance in S, but the underlying relationship exists from first principles. If there is some association between stock size and environmental conditions, then the causal pathway must be environment $\rightarrow$ stock size rather than the reverse. In this case, the expected recruitment becomes $E(R) = E(\text{Egg}) \times E(S) + \text{cov}(\text{Egg}, S)$. The lower bound on the covariance is $-\text{cov}(\text{Egg}, S) > E(\text{Egg}) \times E(S)$ because $E(R) \geq 0$. If the covariance is constant over all levels of S and Egg, then $E(R) = a + b \times E(\text{Egg})$. Once again, recruitment is proportional to egg production despite the influence of the environment on stock size. As Egg $\rightarrow \infty$, $R \rightarrow \infty$, which is impossible; therefore, some form of density dependence must mediate the relationship between stock and recruitment. The null hypothesis that $E(R)$ is constant over all possible stock sizes is equally implausible because it implies that $S \rightarrow \infty$ when Egg $\rightarrow 0$. From first principles, this hypothesis must be rejected, although it may be statistically indistinguishable from a Beverton–Holt stock–recruitment curve with a very steep slope at the origin.

Regardless of how the problem is approached, the hypothesis that no stock–recruitment relationship exists has no basis in logic or biology. At best, it is an impediment to understanding the dynamics of populations that confuses statistical significance with biological reality. At worst, it diverts attention away from essential issues in fisheries science such as sustaining fisheries in the face of an ever-increasing human population.

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ERRATUM

Erratum: Age-specific natural mortality rates of adult Pacific herring (*Clupea pallasi*) from southern British Columbia

R.W. Tanasichuk

Ref: Can. J. Fish. Aquat. Sci. **57**: 2258–2266 (2000).

On page 2260, in the first paragraph under the heading "Natural mortality", the author made an error during his revisions to the galley proofs. Below is the corrected passage.

I estimate M using data for 1980–1998. Figure 4 shows the scatterplot of M against age for age-4 and older fish. Some estimates of M must have been biased because they were < 0. For the West Coast Vancouver Island stock, 29 of the 144 observations had $M < 0$; 20 instances were from older fish (age > 6) when sample sizes were small ($n < 10$), and the remainder were from the sampling years 1989, 1992, 1995, and 1996. For Strait of Georgia herring, 22 of the 137 observations had $M < 0$; three of these were isolated events, seven cases were for older fish when sample sizes were small, and 12 were from 1982, 1989, and 1992.

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R.W. Tanasichuk. Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada (e-mail: tanasichukr@pac.dfo-mpo.gc.ca).

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Are all the tables and figures needed and organized to facilitate comparison? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data depository?

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Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and methods

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Limit the results to answers to the questions posed in the purpose of the work, and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made so as to avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or

unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgements

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. **Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence.** Nearly every manuscript submitted to the Journal contains errors in the references.

Footnotes

Footnotes to material in the text should not be used unless they are unavoidable, but their use is encouraged in tables. Where used in the text, footnotes should be cited in the manuscript by superscript Arabic numbers (except in the tables, see below) and should be numbered serially beginning with any that appear on the title page. Each footnote should be typed on the manuscript page on which the reference to it is made; **footnotes should not be included in the list of references**.

Equations

Equations should be clearly typed; triple-spacing should be used if superscripts and (or) subscripts are involved. Superscripts and subscripts should be legible and carefully placed. Distinguish between lowercase *l* and the numeral *one*, and between capital *O* and the numeral *zero*. A letter or symbol should represent only one entity and be used consistently throughout the paper. Each variable must be defined in the text or in a **List of symbols** to appear after the reference list. Variables representing vectors, matrices, vector matrices, and tensors must be clearly identified. Numbers identifying equations must be in parentheses and placed flush with the **left margin**. In numbering, no distinction is made between mathematical and chemical equations.

Tables

Tables are used to present repetitive data and should be as economical of space as possible. Design tables to fit a one- or two-column width of the Journal. Type each on a separate page and number with Arabic numerals. Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra space instead. Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own. Indicate table footnotes by superscript lowercase letters and type them below the table. Place the tables after the list of references. Note that *text tables* are not numbered, are typed within the text, and seldom need horizontal lines.

Appendices

Figures and tables used in an appendix should be numbered sequentially but separately from those used in the main body of the paper, for example, Fig. A1, Table A1, etc.

Supplemental material

The National Research Council of Canada maintains a depository in which supplementary material may be placed, either at the request of the author or suggestion of the Editors. Such material may include extensive tables of data, detailed calculations, and maps not essential for understanding and evaluating the paper. Such material must be clearly marked when the manuscript is submitted. Tables and figures should be numbered in sequence separate from those published with the paper (e.g., Fig. D1, Table D1). The supplemental material should be referred to by footnotes. Copies of material in the depository may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

Illustrations

Provide photographic reproductions, laser printouts, or the original artwork (no larger than 27.9 × 43.2 cm (11 × 17 in.)) of each illustration. Provide three sets of illustrations for review purposes.

Each figure or group of figures should be planned to fit, after appropriate reduction, into the area of either one or two columns of text. The maximum finished size of a one-column illustration is 8.8 × 23.9 cm (3.5 × 9.4 in.) and that of a two-column illustration is 18.2 × 23.9 cm (7.2 × 9.4 in.). The figures (including halftones) must be numbered consecutively in Arabic numerals, and each one must be referred to in the text and must be self-explanatory. All terms, abbreviations, and symbols must correspond with those in the text. Only essential labelling should be used, with detailed information given in the caption. Each illustration must be identified by the figure number and the authors' names on the back of the page or in the left-hand corner, well away from the illustration area.

Line drawings should be made with black ink or computer-generated in black on high-quality white paper or other comparable material. For computer-generated graphics, supply a laser print at the highest resolution available. **Photocopies are not acceptable.**

All lines must be sufficiently thick (0.5 points minimum) to reproduce well, and all symbols, superscripts, subscripts, and decimal points must be in good proportion to the rest of the drawing and large enough to allow for any necessary reduction without loss of detail. Avoid small open symbols; these tend to fill in upon reproduction. **Lettering produced by dot matrix printers or typewriters, or by hand, is not acceptable.** The same font style and lettering sizes should be used for all figures of similar size in any one paper.

Maps must have very clear, bold patterns and must show longitudes and latitudes (or UTM coordinates) and a scale. All place names and geographic features on Quebec maps must be in French only, with proper accents and capitalization.

Photographs should be *continuous tone* on glossy paper. Prints must be of high quality, on glossy paper, with strong contrast. The copies for reproduction should only show essential features, be trimmed, and mounted on **thin** flexible white bristol board with no space between those arranged in groups. A photograph, or group of them, should be planned to fit into the area of either one or two columns of text **with no further reduction**. Electron micrographs or photomicrographs should include a scale bar directly on the print. The best results will be obtained if the authors match the contrast and density of all figures arranged as a single plate.

Color illustrations will be at the author's expense. Further details on prices are available from Cecily Pearson, Managing Editor of the Journal (613-993-9099; fax: 613-952-7656; e-mail: cecily.pearson@nrc.ca).

NRC Research Press prefers the submission of electronic illustration files for accepted manuscripts and will use these electronic files whenever possible. If electronic files are not available, paper versions of the figures will be scanned. Note that the scanner will easily reproduce flaws (e.g., correction fluid, smudges). Submission of noncontinuous (screened) photographs and scanned illustrations printed out on laser printers is not recommended as moirés develop; a moiré is a noticeable, unwanted pattern generated by rescanning or rescreening an illustration that already contains a dot pattern.

Preparation of electronic illustration files

Authors must supply copies of the original electronic files (i.e., the files directly from the software application that they were created in) and **high-quality laser proofs** of the images. **Electronic files (i.e., figure number and figure content) should match the laser proofs.** On the disk label, identify (1) the software application and version; (2) file name(s), size, and extension; and (3) if the files have been imported from another software. If you have compressed your files, indicate what compression format was used. PC or Macintosh versions of True Type or Type 1 fonts should be used. **Do not use bitmap or nonstandard fonts.** Electronic graphics can be accepted on the following disks: 3.5-in. disks, 100-MB Zip cartridge, and CD-ROM.

All figures should be submitted at their final published size. For figures with several parts (e.g., *a*, *b*, *c*, *d*, etc.) created using the same software application, assemble them into one file rather than sending several files.

Remember that the more complex your artwork becomes, the greater the possibility for problems at output time. Avoid complicated textures and shadings, especially in vector illustration programs; this increases the chance for a poor-quality final product.

Bitmap (raster) files—Bitmaps are image files produced using a grid format in which each square (or pixel) is set to one level of black, colour, or grey. A bitmap (rasterized) file is broken down into the number of pixels or picture elements per inch (ppi). Pixels per inch is sometimes referred to as dots per inch (dpi). The higher the resolution of an image, the larger the number of pixels contained within the rectangular grid.

The proper resolution should be used when submitting bitmap artwork. The minimum requirements for resolution are 600 dpi for line art and finelines (line art with fine lines or shading), 300 dpi for halftones and colour, and 600 dpi for combinations (halftones with lettering outside the photo area).

All *colour* files submitted must be as CMYK (cyan, magenta, yellow, and black). These colours are used in full-colour commerical printing. RGB graphics (red, green, and blue; colours specifically used to produce an image on a monitor) will not print correctly.

Vector files—Vector files are image files produced using elements such as lines and shapes. Typically these files are used for *line drawings*.

Bitmaps inside vector files—Bitmaps can be imported into vector/draw applications only for the purpose of adding and overlaying information, lines, text, etc. Bitmaps should not be resized, cropped, rotated, or otherwise manipulated after importing.

The preferred graphic application of NRC Research Press is CorelDraw! For other applications that can be used, see the "Electronic graphics list" at http://researchpress.nrc.ca/cgi-bin/cisti/journals/rp/rp2_prog_e?cjfas_graphics_e.html.

Format conventions

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel (Bq)
calorie (cal)
centimetre (cm)
centimetre, square (cm^2)
centimetre, cubic (cm^3)
centimetres per gram per second ($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb (C)
decimetre (dm)
degree Celcius (°C)
degrees of freedom (df)
gram (g)
hectare (ha)
hertz (Hz)
hour (h)
joule (J)
kilometre (km)
litre (L)
lumen (lm)
lux (lx)
metre (m)
metre, square (m^2)
metre, cubic (m^3)
micrometre (μm)
milligram (mg)
millilitre (mL)
millimetre (mm)
millimetre, square (mm^2)
minute (min)
molar mass (M)
mole (mol)
moles per litre (mol/L, M)
Pascal (Pa)
second (s)
standard deviation (SD)
standard error (SE)
tonne (metric ton) (t)
volt (V)
volume (vol)
watt (W)

Dates

Dates should be written in the sequence day–month–year without internal punctuation (On 9 October 1983 the...).

Reference citations in text

Name-and-year system

The Journal uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared..." or "...were compared (Brown 1983)". If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared..." or "...were compared (Brown et al. 1983)".

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "personal communication".

Unpublished data

If an unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "In press". Only those manuscripts that are in galley or page proof stage or for which there is an acceptance letter can be considered in press. If an article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpublished data" in the text and do not include it in the reference list.

Reference lists

References should be listed at the end of the paper in alphabetical order according to surnames of the first author. References with the same first author are listed in the following order. (1) Papers with **one author only** are listed first in chronological order, beginning with the earliest paper. (2) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (3) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically.

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *CASSI* or *BIOSIS® Serial Sources*) for references.

Journal article

Peterman, R.M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. **39**: 1444–1452.

Entire issue of journal

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Book in a series

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Book not in a series

LeBlond, P.H., and Mysak, L.A. 1978. Waves in the ocean. Elsevier, New York.

Part of book

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. Neil and D.C. Himsworth. Oregon State University Press, Corvallis, Oreg. pp. 203–229.

Corporate author

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C.

Theses

Kutty, M.N. 1999. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

Smith, J.E. 1981. Catch and efforts statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. No. 1032.

Translation

Koike, A., and Ogura, B. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. [Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.]

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h", "min", and "s" are not used because they are symbols for hour, minute, and second in the sense of duration or the length of time. Thus, "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word list

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the Journal's house style for these commonly used terms:

age-class (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

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Revised January 2002

Recommandations aux auteurs

Journal canadien des sciences halieutiques et aquatiques

La Rédaction encourage la soumission d'articles contribuant à l'enrichissement des connaissances des sciences halieutiques et aquatiques. Les articles peuvent porter sur les cellules, les organismes, les populations, les communautés, les écosystèmes ou les procédés qui affectent les systèmes aquatiques. Ils peuvent traiter de disciplines variées y compris la biologie et l'écologie des organismes d'eau douce et d'eau salée, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, les maladies et la gestion. Les articles sont choisis selon la portée et l'importance des nouvelles connaissances ou idées qu'ils contiennent. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux.

Nous invitons les auteurs à soumettre des articles ayant des objectifs précis ou relevant des hypothèses, concepts ou questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. La raison d'être de l'analyse et l'interprétation des résultats doivent être situées dans un vaste contexte disciplinaire ou interdisciplinaire. Les articles de méthodologie et de modélisation doivent inclure des applications et prouver la supériorité de la méthodologie ou du modèle explicité.

La Rédaction décourage la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicable à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; et d'articles rendant compte de l'application de normes sans gain de méthodologie. Les études préliminaires ou fragmentaires, ou dont la pertinence à grande échelle n'est pas démontrée, et les interprétations purement spéculatives sont carrément rejetées.

Les articles doivent être aussi complets que possible. S'il est impossible d'inclure toute les données pertinentes dans un seul article, l'auteur devra inclure des renvois à des articles connexes et soumettre ceux-ci avec son propre article.

Le Journal publie le genre de textes suivants :

Articles—Études de vaste portée qui constituent de nouvelles contributions à la science.

Perspectives—Synthèses, critiques ou réévaluations de concepts ou de paradigmes couramment admis.

Discussions—Commentaire ou réponse sur des sujets récemment abordés dans le Journal.

Communications rapides—Présentations de nouveaux concepts, descriptions de nouvelles méthodologies ou courts textes sur des sujets d'actualité ou controversés.

On peut obtenir les caractéristiques précises de chacun de ces genres sur le site Web du Bureau de la rédaction http://www.uoguelph.ca/~cjfas/.

Processus de publication

Les manuscrits (**en triple**) doivent être soumis aux directeurs scientifiques, *Journal canadien des sciences halieutiques et aquatiques*, University of Guelph, Département de zoologie, Édifice 006, bureau 107, Guelph, ON N1G 2W1, Canada, et accompagnés d'une lettre qui fait état (1) des principaux points et de l'importance du travail, (2) de la participation de tous les auteurs au manuscrit et de leur approbation de ce dernier, (3) de l'assurance que le manuscrit en question n'est pas en cours d'examen aux fins de publication dans une autre revue, (4) de suggestions quant aux arbitres éventuels, (5) des autres manuscrits, y compris de résumés étoffes contenant une information identique ou similaire à celle contenue dans le manuscrit soumis et (6) des numéros de téléphone et de télécopieur ainsi que du courriel et de l'adresse au courant de chaque auteur. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les demi-teintes) jusqu'à ce qu'on les lui demande. Veuillez inclure le texte, les tableaux et les figures du manuscrit sur disquette, en précisant le(s) progiciel(s) et la (les) version(s) utilisés; le logiciel de traitement de textes préférés est Microsoft Word pour **l'examen par les réviseurs**. **Les manuscrits et les illustrations doivent être solidement emballés afin d'être protégés lors de l'expédition.**

Chaque article est habituellement soumis à une évaluation par au moins deux réviseurs avant d'être examiné par les directeurs scientifiques ou les directeurs scientifiques associés. Toutefois, les directeurs scientifiques renverront sans les évaluer tout article qui ne reflète pas la portée ou le caractère du Journal et les articles qui débordent des limites imposées pour la publication sous forme de « résumés approfondis ». On obtiendra les directives à cet égard sur le site Web du Bureau de la rédaction. Les articles soumis aux fins de publication dans un supplément sont soumis au même rigoureux exercice d'examen que les articles des numéros réguliers.

Voici quelques recommandations générales. Les articles ne doivent pas compter plus de 36 pages dactylographiées à double interligne, y compris les références, les tableaux et les figures. L'auteur devrait limiter le nombre de références à 36 et le nombre combiné de tableaux et d'illustrations à 12. Nous encourageons la soumission d'articles brefs, de style clair. Nous accepterons d'examiner les articles plus longs (p. ex. comptant plus de tableaux et de figures que la norme) et les perspectives intensément étayées (c.-à-d. comptant beaucoup de références) pour autant que leur importance et leur interprétation de nouvelles connaissances en justifient la longueur.

On demande habituellement aux auteurs d'examiner les commentaires des réviseurs et de modifier leur texte en conséquence en 90 jours, tout au plus. Faute de respecter ce délai, l'article renvoyé par l'auteur sera traité comme une nouvelle soumission à moins que l'auteur n'ait au préalable dûment communiqué avec le Bureau de la rédaction.

Droits de reproduction—Lorsqu'un article contient de la documentation (tableaux, figures, schémas, etc.) déjà publiée, il incombe à l'auteur d'obtenir **une permission écrite du titulaire de ces droits d'auteur** pour permettre la reproduction de l'information **en format imprimé et électronique**. La lettre d'autorisation doit accompagner le manuscrit soumis, sans quoi le processus de publication pourra être retardé. Cette lettre sera exigée avec toute mention du type « Tirée de... ». Si une illustration est modifiée, de sorte qu'une telle autorisation

n'est pas requise, la formulation doit être explicite : « Modifiée d'après... ». Si l'illustration est reconstituée à partir d'informations tirées d'autres auteurs la mention doit être du même type que pour une référence.

Soumission de la version approuvée—Les auteurs sont priés de soumettre **une copie imprimée ainsi qu'une copie sur disquette de la version approuvée de l'article seulement**. Les fichiers de texte et les fichiers d'illustrations devront être soumis sur des disquettes distinctes. Le nom des auteurs doit être indiqué clairement sur chaque disquette soumise. Le **texte** (y compris les tableaux) devrait être soumis en format de traitement de texte (WordPerfect, Microsoft Word ou TeX est préférable, compatible à IBM ou Macintosh). Les macros en TeX et la documentation pour la préparation des soumissions sont maintenant disponibles à ftp://ftp.tex.ac.uk/tex-archive/macros/latex/contrib/supported/nrc/, ftp://ftp.dante.de/tex-archive/macros/latex/supported/nrc/ et ftp://ctan.tug.org/tex-archive/macros/latex/contrib/supported/nrc/. Il importe d'indiquer le logiciel de traitement de texte utilisé, la version, de même que le type d'ordinateur (IBM ou Macintosh). En ce qui a trait aux **illustrations**, il convient de se reporter à la section « Préparation des fichiers électroniques des illustrations ». Tout article devra être accompagné d'une lettre faisant foi du fait que la version du texte sur disquette reflète exactement la version imprimée soumise.

Épreuves en placard—Une épreuve en placard, des épreuves des illustrations, une copie de l'article corrigé et un bon de commande de tirés à part sont envoyés à l'auteur correspondant. **Les épreuves en placard doivent être relues attentivement car elles ne sont pas corrigées par les Presses scientifiques du CNRC.** Elles doivent en outre être retournées dans les 48 h suivant leur réception. Au stade des épreuves, il n'est plus temps d'apporter des modifications importantes ni de faire des ajouts ou des suppressions. Les frais occasionnés par des modifications excessives devront être assumés par les auteurs.

Tirés à part—Pour obtenir des tirés à part, il est nécessaire de remplir le bon de commande à cet effet et de le retourner avec le paiement (chèque, numéro de carte de crédit, numéro du bon d'achat ou pièce de journal interne) en même temps que les épreuves et l'article corrigés. Le tarif des tirés à part commandés après la parution du numéro du Journal est fortement majoré. **Le Journal n'offre pas de tirés à part gratuits; ceux-ci ne sont postés qu'après réception d'un numéro du bon d'achat ou du paiement.**

Transfert des droits d'auteur—Tous les auteurs doivent compléter un formulaire de transfert des droits d'auteur au CNRC. On obtiendra ces formulaires auprès du directeur scientifique, dans tout récent numéro du Journal ou sur le site Web des Presses scientifiques du CNRC (http://www.nrc.ca/cisti/journals/).

Toute **permission de republication** partielle ou intégrale d'un article doit être obtenue auprès des Presses scientifiques du CNRC.

Préparation des manuscrits

Directives générales

Le processus de publication sera grandement facilité si les articles sont préparés conformément au style du Journal.

Dactylographiez le manuscrit sur papier blanc (21,6 sur 27,9 cm (8,5 sur 11 po)) sur un côté de la page. Laissez toujours une marge d'au moins 2,5 cm (1 po). Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations. Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à **double interligne**. Si on ne dispose pas d'une police d'italique, souligner les passages à imprimer en italique. En aucun endroit du manuscrit n'écrivez entièrement en majuscules. Pour les références citées dans le texte, utilisez le système nom et année.

Dispositions générales

L'orthographe doit suivre *Le Grand Robert*. Les accents doivent être insérés sur les majuscules. Les auteurs sont responsables de l'uniformité de l'orthographe.

Le *CBE Manual for Authors, Editors, and Publishers : Scientific Style and Format* (6e éd., 1994, Council of Biology Editors, Inc., Chicago, IL 60603, É.-U.) sert de guide quant à la forme de la présentation. Les titres de périodiques sont abrégés suivant *BIOSIS® Serial Sources* (BIOSIS, 2100 rue Arch, Philadelphia, PA 19103-1399, É.-U.). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le Journal suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (5e éd., 1991, Spec. Publ. No. 20, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le Journal suit les recommandations contenues dans Shaklee et al. (1990, Trans. Am. Fish. Soc. **119** : 2–15). Il est recommandé de se servir du **Système international d'unités** ou, tout au moins, de fournir les équivalences en unités SI. Ce système est décrit dans le *Guide de familiarisation au système métrique*, publié en 1989 par l'Association canadienne de normalisation (178, boulevard Rexdale, Etobicoke, ON M6N 3T3, Canada). Pour des raisons pratiques, on permet certaines exceptions à l'utilisation du Système international d'unités (comme dans un éditorial du Journal, vol. 40, n° 12).

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs :

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données?

Si une analyse statistique est effectuée, la statistique cède-t-elle le pas à la recherche? Dans des énoncés de probabilité, les tableaux statistiques inutiles sont-ils exclus et seulement les tests statistiques cités?

Le texte gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le **titre** est-il représentatif du contenu du rapport? Le **Résumé** donne-t-il l'essentiel des connaissances nouvelles? L'**Introduction** se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème? La section **Matériel et méthodes** contient-elle le minimum de détails qui permettront aux lecteurs de saisir les grandes lignes de l'étude et de juger de la valeur des données? Les généralisations sur les **Résultats** sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions? La **Discussion** est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Sont énumérés, ci-dessous, des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

(1) Limiter le résumé à un paragraphe d'environ 175 mots.
(2) Dactylographier à double interligne tous les éléments du manuscrit, y compris la bibliographie et les légendes des tableaux et des figures.
(3) Éviter partout l'emploi exclusif de majuscules, y compris

dans les en-têtes et les légendes des tableaux et des figures.

(4) Ne composer en italique que les noms latins d'organismes et certaines notations statistiques et mathématiques qui devront être imprimés en italique.

(5) Dans les figures, les tableaux et le texte, exprimer les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placer un zéro avant la virgule décimale.

(6) Éviter les formes ambiguës, telles que g C/m^2/jour; utiliser plutôt g C·m^{-2}·$jour^{-1}$.

(7) Identifier le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

(8) Dans le texte, généraliser à partir des tableaux et des figures; éviter une répétition de tous les détails. S'assurer que tous les tableaux et figures sont indépendants et mentionnés dans le texte dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

(9) Préparer la section des remerciements au stade du manuscrit et non à celui des épreuves.

(10) En référant à des communications personnelles, donner les initiales et l'adresse postale de la personne en cause.

(11) Éliminer toutes références qui ne s'appliquent pas directement au problème.

(12) Vérifier soigneusement la correspondance des références en regard des citations dans le texte et vice versa. Dans le cas de matériel difficilement accessible, indiquer l'endroit où le trouver (p. ex., disponible auprès du Département de biologie, Université Laval, Sainte-Foy, QC G1K 7P4, Canada).

(13) Éliminer les virgules entre le nom et la date dans les citations telles que (Smith 1990) et ne pas souligner ni mettre en italique « et al ».

(14) Aux fins de la révision, les photocopies des figures, sauf pour les demi-teintes, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyer des photographies, des imprimés laser ou les dessins originaux/fichiers électroniques des figures (voir « Illustrations » et « Préparation des fichiers électroniques des illustrations »). Utiliser autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organiser le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dresser une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retoucher les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si méthodes, résultats et discussion sont décrits dans des sections séparées.

Organiser les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. S'assurer que ces derniers sont aussi clairs que possible sans référence au texte.

Commencer les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si la conclusion est donnée d'abord puis appuyée ensuite, non seulement le travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voir à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composer la phrase thème de chaque paragraphe et disposer ces phrases dans l'ordre approprié.

Titre

Limiter le titre à ce que l'étude veut démontrer. C'est la clé de l'article et il devrait refléter clairement et brièvement le contenu de l'étude. Le titre a deux fonctions : (1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et (2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance potentielle de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex., rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à... » ou « Recherche sur... ». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires. On évitera les titres en séries.

Résumé

Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquer, au début du résumé, la principale conclusion de l'étude et l'appuyer par des découvertes pertinentes. Ne donner que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et les insérer dans l'énoncé des découvertes. Éviter l'emploi de phrases telles que « ... on y analyse » ou « ... a été trouvé » ; être plus spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir; il importe donc qu'il reflète précisément le contenu du travail et qu'il soit complet en lui-même (c.-à-d. les références absolument *essentielles* sont indiquées, sous forme retraçable, p. ex., R.B. Deriso. 1980. J. Can. Sci. Halieut. Aquat. **37** : 268–282).

Introduction

Limiter l'introduction à la portée, au but et à la raison de l'étude. Ne mentionner que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencer avec le but ou la portée du travail, définir ensuite le problème et ajouter ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375–500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limiter les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si toute l'information est présentée, autant que possible, dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référer aux publications et ne décrire en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : « Afin de déterminer... nous... ». Si la section est longue,

utiliser des sous-titres correspondant à ceux de la section des résultats.

Résultats

Limiter les résultats aux réponses apportées aux questions en rapport avec le but de l'étude; que ces réponses soient aussi complètes que possible, tout en étant concises. Exprimer, autant que possible, les découvertes dans les mêmes termes que les observations ou les mesures, évitant ainsi de confondre faits et spéculations. Dégager les généralités des tableaux et des figures et y référer en évitant de répéter dans le texte ce que les légendes devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries de rapports ou dans un dépôt de données reconnu; et on y référera dans le texte.

Discussion

Limiter la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, les noter et faire ressortir la manière dont les résultats corroborent ou contredisent les travaux déjà publiés. Limiter la spéculation à ce qui peut être appuyé sur des preuves raisonnables. Terminer cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Éviter de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. **On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa.** Presque tous les manuscrits soumis au Journal contiennent des erreurs dans cette section.

Notes infrapaginales

Les notes infrapaginales ne devraient figurer dans le texte que lorsqu'elles sont inévitables, mais leur utilisation dans les tableaux est encouragée. Lorsque les notes infrapaginales figurent dans le texte, leurs appels devraient être désignés par des chiffres arabes en exposant (sauf dans le cas des tableaux, voir ci-dessous) et numérotés consécutivement à partir de la première note infrapaginale, y compris toute note apparaissant en page titre. Chaque note doit figurer au bas de la page où elle est signalée, **pas dans la bibliographie**.

Équations

Les équations doivent être dactylographiées clairement, à triple interligne si elles incluent des exposants ou des indices. Les exposants et les indices doivent être lisibles et correctement placés. Il convient de faire une distinction entre la lettre *l* et le chiffre *un*, et entre la lettre majuscule *O* et le chiffre *zéro*. Une lettre ou un symbole ne devrait représenter qu'une seule entité et devrait être utilisé de façon uniforme dans l'article. Chaque variable doit être définie dans le texte ou dans une **liste des symboles** devant apparaître après la bibliographie. Les variables représentant des vecteurs, des matrices, des matrices vectorielles et des tenseurs doivent être clairement définies. Les numéros d'équation doivent être identifiés par des chiffres arabes placés entre parenthèses et alignés avec la **marge de gauche**. Aucune distinction n'est faite entre la numérotation des formules mathématiques et celle des formules chimiques.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible. Agencez les tableaux de façon qu'ils s'adaptent soit à une ou deux colonnes du Journal. Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes. Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires. Chaque tableau doit avoir une légende instructive, complète et indépendante. Les renvois des tableaux doivent être indiqués par des minuscules suscrites dactylographiées au bas du tableau. Placez les tableaux après la liste des références. Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Annexes

Les illustration et les tableaux en annexe doivent être numérotés différemment des illustrations et tableaux du corps de l'article, p. ex. figure A1, tableau A1, etc.

Documentation complémentaire

Le Conseil national de recherches administre un Dépôt des données non publiées dans lequel la documentation complémentaire peut être versée, à la demande des auteurs ou du directeur scientifique. La documentation complémentaire peut comprendre de grands tableaux, des calculs complexes et des cartes détaillées qui ne sont pas essentiels à la compréhension ou à l'évaluation du document. Les auteurs qui désirent utiliser ce service doivent clairement indiquer les parties dont ils demandent le dépôt dès la soumission de leur article. Les tableaux et les figures doivent être numérotés de façon consécutive, distinctement de ceux destinés à la publication (p. ex. : figure D1, tableau D1). Toute donnée déposée doit faire l'objet d'une note infrapaginale à la section appropriée du document. Des copies des données déposées peuvent être obtenues contre paiement auprès du Dépôt des données non publiées, ICIST, Conseil national de recherches du Canada, Ottawa, ON, K1A 0S2, Canada.

Illustrations

Fournir des photographies, des imprimés laser (les imprimés par imprimante par points sont inacceptables) ou les dessins originaux (pourvu qu'ils ne dépassent pas 27,9 sur 43,2 cm (11 sur 17 po)) de chaque illustration. Transmettre trois copies des illustrations qui serviront au moment de la révision.

Chaque figure ou groupe de figures doit être préparé de manière à occuper, après une réduction appropriée, l'espace d'une ou deux colonnes du texte. Les dimensions finies maximales d'une illustration sur une colonne sont de 8,8 sur 23,9 cm (3,5 sur 9,4 po) et celles d'une illustration sur deux colonnes est de 18,2 sur 23,9 cm (7,2 sur 9,4 po). Les figures (y

compris les similigravures) sont numérotées en chiffres arabes selon leur ordre séquentiel et doivent être annoncées dans le texte bien que tous leurs éléments doivent être intelligibles en eux-mêmes. Tous les termes, les abréviations et les symboles qui se trouvent dans les figures doivent être les mêmes que ceux apparaissant dans le texte. Seuls les détails essentiels devraient être identifiés; l'information plus détaillée devrait paraître dans la légende. Chaque illustration doit être identifiée, de préférence au bas, à gauche, par le numéro de la figure et les noms des auteurs, suffisamment éloignés de l'espace réservé à l'illustration.

Les **dessins au trait** originaux doivent être en encre noire ou produits par ordinateur en encre noire sur du papier blanc de première qualité ou un matériau comparable. Quant aux graphiques produits par ordinateur, il faut fournir un imprimé au laser de la meilleure résolution disponible. **Les photocopies ne sont pas acceptables.**

L'épaisseur des lignes doit être suffisante (au moins 0,5 point) pour que la reproduction soit nette; tous les symboles, les indices supérieurs ou inférieurs et les signes décimaux doivent être en bonne proportion avec le reste du dessin et suffisamment marqués pour permettre la réduction nécessaire sans perte de détail. Ne pas utiliser de petits symboles ouverts qui ont tendance à se refermer lorsqu'ils sont reproduits. **Le lettrage produit par imprimante à matrice de point ou par machine à écrire, ou à la main, ne sont pas acceptables.** Le même style de police et la même dimension de lettrage doivent servir dans toutes les figures de dimensions semblables d'un article.

Les **cartes** doivent avoir des motifs clairs et gras, et comporter les longitudes et latitudes (ou les coordonnées de Mercator transverses universelles) de même qu'une échelle graphique. Tous les noms d'endroits et les caractéristiques géographiques sur les cartes du Québec doivent être en français seulement, avec les accents et les majuscules appropriés.

Les **photographies** doivent être en *demi-teintes* sur papier glacé. Les épreuves doivent être de première qualité, sur papier glacé, avec contrastes bien marqués. Les copies destinées à la reprographie doivent être rognées de façon à ne laisser paraître que les éléments essentiels et être montées sur carton bristol blanc **léger** et flexible. Aucun espace ne doit paraître entre les photographies constituant une même planche. Une photographie ou un groupe de photographies doit être préparé de manière à occuper, **sans réduction supplémentaire**, une ou deux colonnes du texte. Les micrographies électroniques et les photomicrographies doivent comporter une échelle graphique à même l'illustration. On évitera les pertes de détails et de contraste inhérentes au procédé d'imprimerie en agençant le contraste et la densité de toutes les photographies montées sur une même planche.

Les **illustration en couleurs** sont aux frais de l'auteur. On peut obtenir de plus amples renseignements sur les prix auprès de Cecily Pearson, rédactrice administrative du Journal (613-993-9099; télécopieur : 613-952-7656; courriel : cecily.pearson@nrc.ca).

Les Presses scientifiques du CNRC préfèrent recevoir la version électronique des illustrations des manuscrits acceptés et l'utiliseront dans la mesure du possible. Si les fichiers électroniques ne sont pas disponibles, la version papier des illustrations sera balayée. Toutefois, le lecteur optique reproduit facilement les défauts (p. ex., le liquide correcteur, les maculations). Il est recommandé de ne pas soumettre de photographies tramées, ni des illustrations balayées imprimées au laser, à cause des moirures qui se forment; une moirure est un motif perceptible et non désiré produit par le rebalayage ou le retramage d'une illustration qui contient déjà un motif de points.

Préparation des fichiers électroniques des illustrations

Les auteurs doivent fournir des copies des fichiers électroniques originaux (c.-à-d. les fichiers tirés directement du logiciel sur lequel ils ont été créés) et des **épreuves au laser de haute qualité** des images. **Les fichiers électroniques (c-à-d. le numéro et le contenu de la figure) doivent correspondre aux épreuves au laser.** Sur l'étiquette de la disquette, il faut indiquer (1) l'application et la version du logiciel; (2) le(s) noms(s), la dimension et le suffixe du fichier; et (3) si les fichiers ont été importés d'un autre logiciel. Si les fichiers ont été comprimés, il faut indiquer le format de compression. Les versions PC ou Macintosh des polices True Type ou Type 1 doivent être utilisées. **On ne doit pas se servir des polices de caractères en mode points ou non standard.** Les graphiques électroniques sont acceptés sur les disquettes suivantes : disquettes 3,5 po, cartouche Zip de 100 Mo et sur CD-ROM.

Toutes les illustrations doivent être soumises dans leur dimension de tirage final. Quant aux figures comportant plusieurs parties (p. ex., *a*, *b*, *c*, *d*, etc.) crées sur le même logiciel, elles doivent être assemblées dans un seul fichier plutôt que de transmettre plusieurs fichiers.

Il faut **garder** les illustrations le plus simple possible afin d'éviter les embûches. Éviter les textures et les fonds grisés compliqués, particulièrement dans les programmes d'illustration vectorielle.

Fichier en mode point—Les fichiers d'images en mode point sont produits sur une grille dans laquelle chaque carré (ou pixel) correspond à une nuance de noir, de couleur ou de gris. Un fichier en mode point est défini par le nombre de pixels ou d'éléments par pouce. Comme le pixel est défini comme un point, on parle aussi de « points par pouce » ou « ppp ». Plus la résolution de l'image est grande, plus le nombre de points sur une surface donnée est élevé.

Les illustrations soumises en mode point doivent être de définition adéquate. Les exigences minimales de définition sont les suivantes : 600 ppp pour les dessins au trait et les lignes fines (les dessins au trait comportant des lignes fines ou un fond grisé), 300 ppp pour les demi-teintes et les couleurs et 600 ppp pour les combinaisons (demi-teintes avec lettrage à l'extérieur de l'espace réservé à la photo).

Tous les fichiers en *couleurs* soumis doivent suivre la base CMJK (cyan, magenta, jaune et noir). Ces couleurs sont utilisées dans l'impression commerciale tout en couleur. Les graphiques RVB (le rouge, le vert et le bleu sont les couleurs utilisées particulièrement pour produire les images sur un écran) ne s'impriment pas correctement.

Fichiers vectoriels—Les fichiers vectoriels sont des fichiers d'images composées d'éléments comme les lignes et les formes. Ce type de fichier convient habituellement aux *dessins au trait*.

Fichiers vectoriels en mode point—Le mode points peut être importé dans un logiciel vectoriel ou de dessins aux seules fins d'ajouter et de superposer de l'information, des lignes, du texte, etc. Le mode points ne doit pas être redimensionné, recadré, tourné ou manipulé de toute autre façon après avoir été importé.

Le logiciel graphique préféré par les Presses scientifiques du CNRC est CorelDraw! Pour les autres applications possibles, reportez-vous à la « Liste des programmes graphiques électroniques » à http://researchpress.nrc.ca/cgi-bin/cisti/journals/rp/rp2_prog_f?cjfas_graphics_f.html.

Conventions

Abréviations

N'abréger les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

becquerel (Bq)
calorie (cal)
centimètre (cm)
centimètre carré (cm^2)
centimètre cube (cm^3)
centimètres par gramme par seconde ($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb (C)
décimètre (dm)

degré Celsius (°C)
degrés de liberté (dl)
écart type (ET)
électronvolt (eV)
erreur type (ErT)
farad (F)
gramme (g)
hectare (ha)
hertz (Hz)
heure (h)
joule (J)
kilomètre (km)
litre (L)
lumen (lm)
lux (lx)
mètre (m)
mètre carré (m^2)
mètre cube (m^3)
micromètre (µm)
milligramme (mg)
millilitre (mL)
millimètre (mm)
millimètre carré (mm^2)
minute (min)
masse molaire (M)
mole (mol)
moles par litre (mol/L, M)
pascal (Pa)
seconde (s)
tonne (métrique) (t)
volt (V)
volume (vol)
watt (W)

Dates

On écrira les dates dans l'ordre jour–mois–année, p. ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le Journal utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : « Brown (1983) compare... » ou « ...on a comparé (Brown 1983) ». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de « et al. » : « Brown et al. (1983) comparent... » ou « ...on a comparé (Brown et al. 1983) ».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de « communication personnelle ».

Données inédites

Si un livre ou un article inédit a été accepté pour publication, insérez-le dans la liste des références, avec la mention « Sous presse ». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si un article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention « données inédites ».

Liste de références

Les références doivent être classées par ordre alphabétique des noms des premiers auteurs. Si la liste comprend plusieurs publications d'un même premier auteur, les règles suivantes seront appliquées. (1) S'il n'y a qu'**un auteur**, les ouvrages se succèdent par ordre chronologique de partition en commençant par le plus ancient. Si l'auteur a publié, au cours d'une année, plusieurs livres ou articles, on distinguera les références les unes des autres en faisant suivre l'année de publication d'une lettre (*a*, *b*, *c*, etc.). (2) S'il y a **deux auteurs**, les ouvrages sont placés à la suite de ceux n'ayant qu'un auteur, et sont alors classés par ordre alphabétique du nom du deuxième auteur. (3) S'il y a **trois auteurs ou plus**, les ouvrages sont placés à la suite de ceux ayant deux auteurs et sont classés par ordre chronologique.

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après *CASSI* ou *BIOSIS® Serial Sources*).

Article d'une revue

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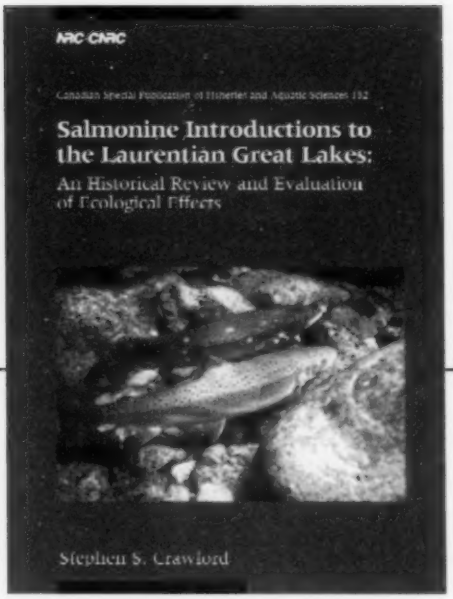
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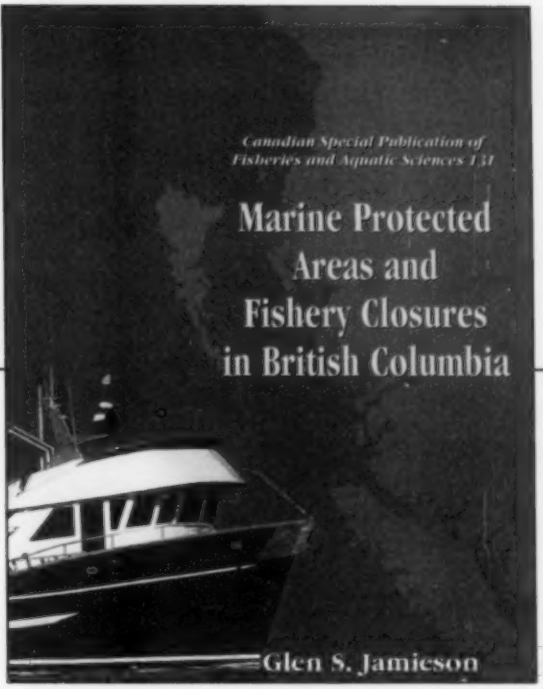
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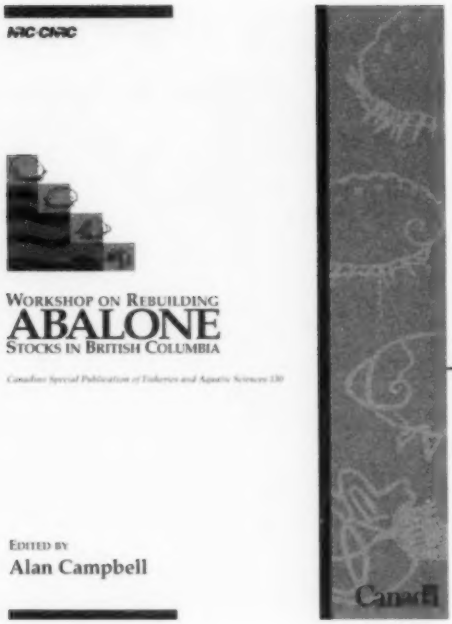

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